II. REMARKS

Claims 1 to 15 are pending in the subject application, were examined and stand variously rejected. Claims 7 to 11 have previously been withdrawn from consideration as a result of a requirement for restriction. By this Amendment, claims 2, 3, 5, 6, and 12-15 have been canceled without prejudice or disclaimer. Claims 1 and 4 have been amended and new claims 16-33 have been added. Applicants' cancellation of claims and the amendment of the claims as previously presented are made without prejudice to Applicants' right to pursue the same or similar claims in a related application. The cancellation of these claims and the amendment of claims 1 and 4 are not intended to be a dedication to the public of the subject matter of the claims as previously presented.

The specification has been amended to insert reference to the sequence identification numbers which in turn correspond to the sequences listed in the attached sequence listing. The identification of the sequences and Applicants' request for insertion of the attached sequence listing does not raise an issue of new matter.

The claim amendments and the addition of new claims 16-33 do not raise an issue of new matter. Support for the amendments is found in the application papers as follows.

Amended claim 1 is supported on page 2, lines 16-24; page 10, lines 5-7; and page 34, line 15 to page 36, line 10.

Amended claim 4 is supported on page 9, lines 30-31.

New claim 16 is supported on page 2, line 30.

New claim 17 is supported on page 2, line 30.

New claim 18 is supported on page 2, line 30.

New claims 19 and 24 are supported on page 9, lines 21-31 and page 35, line 15 to page 36, line 5.

New claims 20 and 25 are supported on page 2, line 30.

New claims 21 and 26 are supported on page 2, line 30.

New claims 22 and 27 are supported on page 2, line 30.

New claims 23 and 28 are supported on page 9, lines 30-31.

New claim 29 is supported on page 9, lines 21-31; page 10, line 5 and page 26, lines 1-5.

New claim 30 is supported on page 9, lines 30-31 and page 26, lines 1-5.

New claim 31 is supported on page 35, lines 22-29 and page 26, lines 1-5.

New claim 32 is supported on page 35, lines 22-29 and page 26, lines 1-5.

New claim 33 is supported on page 35, lines 22-29 and page 26, lines 1-5.

Entry of these amendments is respectfully requested.

This Amendment changes, adds and deletes claims in this application. A detailed listing of all claims that are, or were, in the application, irrespective of whether the claim(s) remain under examination in the application, is presented, with an appropriate defined status identifier.

In view of the preceding amendments and the remarks which follow, reconsideration and withdrawal of the rejections is respectfully requested. After

amending the claims as set forth above, claims 1, 4 and 16-33 are presently pending and under examination.

Election/Restrictions

The Office acknowledged Applicants' election without traverse of Invention I and the species of fluoropyrimidine, colon/colorectal cancer and a polymorphism or genotype ERCC1, in the reply filed September 9, 2007. Claims 1-6 and 12-15 have been examined to the extent they read on the elected subject matter which Applicants have interpreted to encompass the elected species. Upon an indication of the allowance of the elected species, the Office will expand the search to include other species.

Applicants also note that newly added claims are directed to the subject matter of the restricted class and elected species. Elected Invention I is directed to methods for selecting a therapeutic regimen for treating a cancer by screening for a genomic polymorphism or genotype and the species election of a fluoropyrimidine, colon cancer/colorectal cancer and ERCC1. Amended claim 1 is directed to selecting a therapy comprising the administration of a fluoropyrimidine and a platinum drug to a human metastatic colorectal cancer patient by screening a cell or tissue sample isolated from the patient for the genotype at codon 118 of the ERCC1 gene, wherein the genotype (C/C) selects the human patient for treatment with said therapy. New claims 16 -18 further define claim 1 by selecting the fluoropyrimidine to be 5-Fluorouracil and/or the platinum drug to be oxaliplatin or further comprising radiation therapy. New claim 19 is directed to a method for determining whether a human metastatic colorectal cancer patient is more likely responsive to therapy comprising the administration of a fluoropyrimidine and a platinum drug by screening a cell or tissue sample isolated from the patient at codon 118 of the ERCC1 gene, wherein the presence of the genotype (C/C) at codon 118 of the ERCC1 gene indicated the patient is more likely responsive

to said therapy. New claims 20 to 23, are dependent on claim 19 and further define the claim by selecting the fluoropyrimidine to be 5-Fluorouracil and/or the platinum drug to be oxaliplatin and further wherein the therapy comprises radiation. New claim 24 is to a method for determining whether a human metastatic colorectal cancer patient is less likely to experience longer survival following treatment comprising the administration of a fluoropyrimidine and a platinum drug, comprising screening a cell or tissue sample isolated from said patient containing codon 118 of the ERCC1 gene, wherein the presence of the genotype (C/T) or (T/T) indicates said patient is less likely to experience longer survival following treatment with said therapy. New claims 25 to 28 further define claim 24 by indicating a specific fluoropyrimidine and the platinum drug, and further comprising radiation therapy. New claim 29 is directed to a method for treating a human metastatic colorectal cancer patient by administering an effective amount of a therapy comprising a fluoropyrimidine and a platinum drug to a patient selected for the therapy based on the possession of the genotype (C/C) at codon 118 of the ERCC1 gene. Claims 30 to 33 are dependent on claim 29 and further define the claim by selecting the fluoropyrimidine to be 5-Fluorouracil and/or the platinum drug to be oxaliplatin, or further defining the therapy to include radiation therapy.

All of the above claims are within the elected Invention of group I and the species election of a therapy comprising a fluoropyrimidine, metastatic colorectal cancer and ERCC1.

Claim Objections

The Office objected to claims 2 through 6 for allegedly including subject matter of the non-elected inventions, namely the alternative therapy of a platinum drug (claims 2 and 4), the additional types of cancer (claim 3) and the additional genes (claims 5 and 6). The Office has also objected to claim 3 for allegedly referring to the same species by two different names – i.e., colon cancer and colorectal cancer. Claim 12 was objected to for a typographical error.

Applicants respectfully traverse for the following reasons. With respect to the inclusion of non-elected species within the generic claim, 37 C.F.R. §§ 1.141 and 1.146 do not require amendment of the generic claims to the specific examined species. Pursuant to MPEP § 809.02(a), "[u]pon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141." Nevertheless, Applicants have amended the claims to recite metastatic colorectal cancer and the polymorphism present at position 118 of the ERCC1 gene. Applicants reserve their right to have the full scope of the generic claim as previously presented examined after the present species claim has been found allowable.

With respect to the typographical error in claim 12, the claim has been canceled without prejudice or disclaimer thereby removing this ground for objection. In view of these amendments, reconsideration and withdrawal of the objections are respectfully requested.

Specification

The Office objected to the specification because the assigned SEQ ID NOs allegedly have not been used to identify each sequence listed. In response, the

specification has been amended as required by the Office and a paper copy and computer readable form of a Sequence Listing has been provided. In view of these amendments, reconsideration and withdrawal of the objection is respectfully requested.

35 U.S.C. § 112, Second Paragraph

Claims 1 to 6 and 12 to 15 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter regarded as invention.

Claims 1 to 6 and 12 to 15 stand rejected as allegedly indefinite for not reciting a clear nexus between the preamble of the claims and the final process step of the claims. Claim 4 was objected to for use of the recitation of the term "the cancer treatment" because the phrase lacked proper antecedent basis. Claim 6 was alleged indefinite for use of the recitation of the term "corresponds." Claims 6 and 14 were alleged to be indefinite for use of the term "the genotype is high expression of a gene."

Without conceding the correctness of the Office's position and in a sincere effort to advance examination and allowance, the claims have been amended thereby obviating this ground for rejection. Reconsideration and withdrawal of these rejections are therefore respectfully requested.

35 USC § 112 , First Paragraph – Written Description

Claims 1 to 6 and 12 to 15 stand rejected under 35 USC § 112, first paragraph, as containing subject matter which allegedly was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed.

In sum, the Office stated that the claims exceed the exemplified examples provided in Applicants' specification, with respect to genotype being tested, the polymorphism being tested within the ERCC1 gene, the taxonomic identity of the patient sample (e.g., the gene could be in any of human or non-human organism), and the correlation between the polymorphism and the treatment.

Applicants respectfully traverse; however, the claims have been amended without prejudice or disclaimer to a scope of which is supported by the specification as filed. Independent claim 1 and dependent claims 4 and 16-18 are supported in the specification on page 2, lines 16-24; page 10, lines 5-7; page 34, line 15 to page 36, line 10; page 9, lines 30-31; page 2, line 30; and page 35, lines 22-29. New independent claims 19 and 24 and dependent claims 21-23 and 25-28 are supported on page 9, lines 21-31; page 35, line 15 to page 36, line 5; page 2, line 30; page 9, lines 30-31; page 10, lines 5-7; page 35, lines 22-29; page 9, lines 24-27 and page 35, line 30 to page 36, line 5. New independent claim 29 and dependent claims 30-33 are supported on page 9, lines 21-31; page 10, line 5; page 26, lines 1-5 and page 34 line 15 to page 36 line 10. The claims are now directed to the use of a single polymorphism (C→ T at position 118) in a singe gene (the ERCC1 gene), in a single species (a human metastatic colorectal patient) to determine likelihood of a specified therapy or selecting an appropriate therapy (the therapy comprising administration of a fluoropyrimide and a platinum drug).

In view of the claim amendments and their specific support in the specification, reconsideration and withdrawal of the rejection of the claims under 35 U.S.C. § 112, first paragraph, written description, is respectfully requested.

35 U.S.C. § 112, First Paragraph – Enablement

Claims 1 to 6 and 12 to 15 stand rejected under 35 U.S.C. § 112, first paragraph, because the specification, while allegedly being enabling for methods for predicting the survival of a human patient having metastatic colon cancer, the method comprising: i) obtaining a nucleic acid sample from colon cancer tissue or colon cancer cells of a human patient having metastatic colon cancer or colon cancer cells of a human patient having metastatic colon cancer and treated with 5-fluoropyrmidine (5-FU) and oxaliplatin, wherein the nucleic acid sample comprises ERCC1 nucleic acids; ii) analyzing the sequence of the ERCC1 nucleic acids to determine the nucleotides present at codon 118; and iii) determining that the patient will have a longer survival following treatment with 5-FU and oxaliplatin if the patient has a C/C genotype at codon 118 of ERCC1, as compared to patients having a C/T or T/T genotype at codon 118 of ERCC1, allegedly does not reasonable provide enablement for methods which select any therapeutic regimen in any subject having any type of cancer by assaying for any polymorphism or genotype of the ERCC1 gene or any other gene. The Office alleged that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The Office concluded that, in the instant case, the specification did not enable one of skill in the art to practice the invention as it is broadly claimed because the specification allegedly does not teach a representative number of polymorphisms or genotypes in the ERCC1 gene or in other genes of the human genome or in the genomes of non-human organisms which are correlated with response to any type of cancer therapy.

Applicants respectfully traverse, however, the claims have been amended to recite a method for selecting a therapy comprising the administration of a

fluoropyrimidine and a platinum drug for treating a human metastatic colorectal patient, comprising screening a cell or tissue sample isolated from the patient for the genotype at codon 118 of the ERCC1 gene, wherein the genotype (C/C) selects the human patient for treatment with said therapy. New claims 19 and 24 recite a method for determining whether a human patient having a specified cancer is more likely or less likely to exhibit longer survival following treatment comprising the administration of a fluoropyrimidine and a platinum drug by screening a cell or tissue sample isolated from the patient for the genotype at codon 118 of the ERCC1 gene, wherein the presence of the genotype (C/C) at codon 118 of the ERCC1 gene indicated the patient is more likely responsive to said therapy. New claim 29 recites a method for treating a human metastatic colorectal cancer by administering an effective amount of a therapy comprising a fluoropyrimidine and a platinum drug to a patient selected for the therapy based on the possession of the genotype (C/C) at codon 118 of the ERCC1 gene in a sample isolated from the patient.

With respect to the Office's allegation that the specification only enables treatment with 5-fluoropyrmidine (5-FU) and oxaliplatin, Applicants submit that the currently pending claims 1, 4 and 16-33 now recite a therapy comprising a fluoropyrimidine and a platinum drug. 5-fluoropyrimidine (5-FU) and oxaliplatin are specific chemotherapeutic drugs of these classes which are known to treat metastatic colorectal cancer. The link among the members of these drug classes is the similar chemical structures and mechanisms of action. For example, 5-fluorouracil (5-FU) is a pyrimidine analog and an antimetabolite chemotherapeutic anticancer agent. It has been in used against cancer for about 40 years, acts in several ways, but principally as a thymidylate synthase inhibitor, interrupting the action of an enzyme which is a critical factor in the synthesis of pyrimidine which is important in DNA replication. Papamicheal (1999) The Oncologist **4**:478-487 (Exhibit **A**) describes many equivalents to 5-FU including prodrugs, analogs and derivative thereof. Oxaliplatin is a platinum-based

chemotherapy drug in the same family as cisplatin and carboplatin. Platinum-based chemotherapy drugs work by cross-linking subunits of DNA thereby disrupting the normal cell cycle by impairing normal DNA synthesis, transcription, and function as described in Wiseman et al. (1999) Drugs Aging **14**(6):459-475 (Exhibit **B**). Therefore, in view of this knowledge in the art and the teachings of the specification, Applicants submit that the specification, in combination with the knowledge available at the time the application was filed, enables the use of the ERCC1 polymorphism at position 118 to predict responsiveness to therapy comprising the administration of a fluoropyrimidine and a platinum drug to a human patient.

The Office also alleged that the specification is enabling only for methods for predicting the survival of a human patient having metastatic colon cancer and not the broad range of cancers as claimed. The currently pending independent claims 1, 19, 24 and 29 and their respective dependent claims now recite human metastatic colorectal cancer patients.

Applicants also submit that the amended claims are not contradicted by the Office's allegations of unpredictability in support of the enablement rejection. For example, the Office cited Hirschhorn et al. (2002) Genetics in Medicine **4**(2):45-61 for teaching that genetic variants and phenotypes are not robust associations leading to unpredictability in the area of polymorphism and phenotypes. "Hirschhorn states that 'of the 166 putative associations studied three or more times, only 6 have been consistently replicated.'" Applicants respectfully disagree with the generalization of this study to the now claimed predictive association of the genotype at codon 118 of the ERCC1 gene and selected cancer patient's responsiveness to treatment comprising a fluoropyrimidine and a platinum drug. Hirschhorn et al. generalizes polymorphism associated with common diseases and their strength of association. This review did not evaluate any studies associating ERCC1 polymorphisms and cancer treatment or

studies regarding predicting a selected cancer patient's responsiveness to a fluoropyrimidine and a platinum drug therapy.

The Office cited Winter (2005) Oncogene **24**:2110-2113 for teaching the unpredictability of additional variants of ERCC1, specifically a mutation in Exon 1 of the ERCC1 gene, and its correlatation with response to treatment. As Applicants now claim a different polymorphism in the ERCC1 gene, the teachings of this reference are irrelevant to the claimed invention. The teachings of Winter applied to a mutation in Exon 1, not the now claimed polymorphism of codon 118, in Exon 4, of the ERCC1 gene.

The Office cited Yu et al. (Mutation Research (1997) **382**:13-20) and Kang (Experimental and Molecular Medicine (2006) **38**:320-324) to show the unpredictability of extrapolating the results obtained with the codon 118 polymorphism to other types of outcomes, chemotherapeutic agents and cancers. However, the teaching of Yu et al. and Kang are directed to predicting sensitivity of ovarian cancer tissues or patients suffering from ovarian cancer to platinum-based drugs. Applicants' claims now recite metastatic colorectal cancer and therefore, the reference does not support the failure of the specification to support the claimed invention.

The Office alleged that several teachings related to gene expression or protein levels of ERCC1 showed unpredictable in the art for predicting patient response to a variety of platinum based chemotherapy treatments, see Lee (2005) Proceedings American Association Cancer Research **46**: Abstract 1496; Viguier et al. (2005) Clinical Cancer Research **11**:6212-6217; Yu (2000) Cancer Letters **151**:127-132; and Britten (2000) International Journal of Cancer **89**: 453-457. The currently pending claims 1, 4, 12 and 16-32 are directed towards evaluating the genotype at codon 118 of the ERCC1 gene and are thus not directed to evaluating gene expression or protein levels of ERCC1. Thus, these references fail to establish that the specification, in combination

with the knowledge available to the skilled artisan, enable the full scope of the amended claims.

The Office cited Grau (2005) Journal of Clinical Oncology **23**.511S, for allegedly teaching that in oropharyngeal carcinoma patients, the ERCC1 118 codon polymorphism was not correlated with response to cisplatin/5-FU therapy. Applicants respectfully disagree with the assertion that the ERCC1 polymorphism evaluated in the teachings of Grau are directed to the ERCC1 118 codon. The polymorphism evaluated by Grau is "ERCC1 (Lys259Thr)" which is a Lysine to Threonine mutation at amino acid 259. As described in the specification on page 35, lines 15-18, the C to T single nucleotide change at codon 118 of the ERCC1 gene results in the same asparagine amino acid transcribed from two different codons (AAC) versus (AAT).

The Office alleged the unpredictability of extrapolating the results obtained with the codon 118 polymorphism to response to other chemotherapies citing the post-filing date art of Viguier et al. (2005) Clinical Cancer Research **11**:6212-6217. Viguir allegedly teaches that the T/T genotype of the ERCC1 codon is associated with a response to metastatic colorectal cancer patients receiving 5-FU/oxiplatin treatment. The Office also alleged that Viguier et al. (2005) Clinical Cancer Research **11**:6212-6217 teaches the T/T, C/T, and C/C genotypes were not associated with response to 5-FU alone or 5-FU in combination with irinotecan. Applicants submit that Viguier et al. (2005) did not measure survival time following therapy and therefore not Applicants' claimed endpoint. (See page 6213, right hand column under "Chemotherapy Response Criteria.") Thus, the alleged results reported in Viguier et al. (2005), are not within the scope of the amended claims.

The Office also alleged that it is unpredictable as to whether the results obtained with humans can be extrapolated to other organisms as described in Halushka (1999) Nature **22**: 239-247. Without acquiescing that the predictive genotype at codon 118 of

the ERCC1 gene can not be extrapolated to other organisms and in an effort to further prosecution, the currently pending independent claims 1, 20, and 27 are limited to a "human metastatic colorectal cancer patient."

The Office alleged that it is unpredictable as to whether samples other than intratumoral colorectal cancer samples can be analyzed to predict survival following 5-FU/oxiplatin therapy. The Office argued that it is well known in the art that mutations arise spontaneously in tumor samples, such that a polymorphism present in normal tissue may not be present in tumor tissue and vice versa. Applicants respectfully disagree, as shown in Shen et al. (1998) Cancer Res. **58**:604-608 (Exhibit **C**) the C to T polymorphism at codon 118, in Exon 4 of the ERRC1 gene can be found in normal healthy individuals at a frequency of 0.46 (see Table 2 on page 605). Thus, the teachings of the specification in combination with the knowledge available to the skilled artisan, enables the scope of the amended claims.

Accordingly, Applicants submit that the specification, in combination with the knowledge available to the skilled artisan, fully enables the full scope of the amended claims and therefore, reconsideration and removal of the rejections under 35 U.S.C. § 112, first paragraph (enablement) is respectfully requested.

Double Patenting

Claims 1 to 6 and 12 to 15 stand provisionally rejected on the ground of nonstatutory obvious-type double patenting as allegedly unpatentable over claims 1 to 7 and 14 to 22 of copending Application No. 11/173,889. The Office conceded that the conflicting claims are not identical, but alleged that they are not patentably distinct from each other because the present claims and the claims of '889 are both inclusive of methods for selecting a therapeutic regimen for treating cancer comprising screening a cell or tissue sample from a patient for a genomic polymorphism or genotype. In

particular, the Office alleged that the present claims and the claims of '889 are both inclusive of methods in which the cancer is colon cancer and the treatment is 5-FU and oxaliplatin. The Office further conceded that while the claims of '889 do not specifically recite the detection of the codon 118 C to T polymorphism in ERCC1, when read in light of the specification of '889, allegedly it is clear that the broad recitation of any polymorphism or genotype is intended to encompass the codon 118 C to T polymorphism in the ERCC1 gene.

Claims 1 to 4, 12 and 15 stand provisionally rejected on the ground of nonstatutory obviousness-type double patenting as allegedly unpatentable over claims 47, 48, 52, 53, 56, 61-66 and 68-70 of copending Application No. 09/715,764. The Office conceded that the conflicting claims are not identical, but alleges that they are not patentably distinct from each other. The present claims are drawn generically to methods for selecting a therapeutic regimen for treating cancer comprising screening a cell or tissue sample from a patient for a genomic polymorphism or genotype. The claims of '764 are drawn to methods for identifying colorectal cancer patients sensitive to TS-directed chemotherapy by assaying for the polymorphism of double repeat of the 28bp repeat sequence in the 5 'UTR of the TS gene. The Office argued that the claims of '764 are drawn to methods for detecting a polymorphism which is correlated with response to cancer therapy, as is allegedly encompassed by the present claims. The Office further alleged that, thereby the present claims are generic to all that is recited in the claims of '764.

Claims 1 to 4, 12 and 15 stand provisionally rejected on the ground of non-statutory obvious-type double patenting as allegedly unpatentable over claims 1-20 of copending Application No. 11/681,670 (Applicants believe that the Office has made a typographical error when reciting Application No. 11/691,670 and therefore Applicants reviewed the rejection with respect to Application No. 11/681,670). The Office

conceded that the conflicting claims are not identical, but alleges that they are not patentably distinct from each other. The present claims are drawn generically to methods for selecting a therapeutic regimen for treating cancer comprising screening a cell or tissue sample from a patient for a genomic polymorphism or genotype. The Office stated that the claims of '670 are drawn to methods for identifying patients suffering from gastrointestinal cancer that is suitably treated with a therapy comprising assaying for the presence of SCN1A_T107\67A_SNP T/T polymorphism in the VGSC gene as indicative of a suitable response to therapy. The Office further argued that the claims of '670 are drawn to methods for detecting a polymorphism which is correlated with response to cancer therapy, as is allegedly encompassed by the present claims. The Office argued that allegedly the present claims are generic to all that is recited in the claims of '670.

Claims 1 to 4, 12 and 15 stand provisionally rejected on the ground of nonstatutory obviousness-type double patenting as allegedly unpatentable over claims 1 to 11 of copending Application No. 11/681,695 (Applicants believe that the Office has made a typographical error when reciting Application No. 11/681,615 and therefore Applicants reviewed the rejection with respect to Application No. 11/681,695). The Office conceded that the claims are not identical, but alleges that they are not patentably distinct from each other. The Office stated that the present claims are drawn generically to methods for selecting a therapeutic regimen for treating cancer comprising screening a cell or tissue sample from a patient for a genomic polymorphism or genotype. The Office further stated that the claims of '695 are drawn to methods for identifying patients having cancer that will respond to anti-VEGF based chemotherapy by assaying for a polymorphism or genotype selected from IL-18 – 251, VEGF 936, and AM 3' CA repeats. The Office alleged that the claims of '695 are drawn to methods for detecting a polymorphism which is correlated with response to cancer therapy, as is

encompassed by the present claims. The Office further alleged that the present claims are generic to all that is recited in the claims of '695.

Applicants respectfully defer responding to the provisional grounds of these rejections until allowable subject matter has been indicated in one or more of the allegedly conflicting applications.

35 U.S.C. § 102

Claims 1 to 6 and 12 to 15 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Yu et al. (Mutation Research, (1997) 382: 13-20). The Office argued that in the instant application, the claim language of "selecting a therapeutic regimen for treating a cancer" is allegedly a statement of purpose and intended result and does result in a manipulative difference in the method steps of the claims. The Office interpreted the claims as allegedly being limited to methods comprising screening a cell or tissue sample from a patient for a genomic polymorphism or genotype, wherein it is a property of the polymorphism or genotype that is correlated with treatment outcome of cancer.

The Office stated that Yu teaches a method comprising obtaining a tissue sample (ovarian caner tissue and normal ovarian tissue) from a patient having ovarian cancer and assaying the tissue sample for the presence of a T or a C at codon 118 in exon 4 of the ERCC1 gene. The Office argues that, therefore Yu allegedly anticipates the claimed invention.

The Office stated that, regarding claim 2, ovarian cancer is a cancer that can be treated with fluoropyrimidine.

The Office argued that, regarding claims 3, and 2 through 15, the preamble of the claims is not considered to further limit the claims and that the claims do not require

that the patient from which the sample has been isolated has colon cancer. The Office further states that, regarding claim 4, ovarian cancer is a cancer that can also be treated with radiation therapy.

With regards to claims 5, 13 and 15, the Office alleged that Yu teaches that the 118 C to T polymorphism is detected in the ERCC1 gene.

With regards to claims 6 and 14, the Office stated that the claims allegedly do not set for the relationship between the polymorphism and high expression of a gene. The Office argued that a genotype is allegedly considered to be limited to an allelic composition of a cell or gene. It is further alleged that the claims do not require performing a step in which high gene expression is detected by, for example, determining the quantity of a mRNA. The Office interpreted the claims to be inclusive of a method for detecting a polymorphism or genotype, wherein the polymorphism or genotype is correlated with high expression of a gene, presently considered to be a property of the ERCC1 C to T polymorphism that is associated with high expression of ERCC1.

With regards to claims 12 and 15, the Office alleged that the claims do not require an active step of administering 5-FU/oxaliplatin and do not require a specific step in which 5-FU/oxaliplatin is selected for treatment. The Office concluded that the recitation that the therapeutic regimen comprises administering 5-FU and oxaliplatin is allegedly not considered to further limit the claims.

Further regarding claim 15, the Office considered it to be a property of the ERCC1 C to T polymorphism at codon 118 that is associated with the probability of recurrence free survival.

Applicants respectfully traverse; however, the claims have been amended to recite a method for selecting a therapy comprising the administration of a fluoropyrimidine and a platinum drug to a human metastatic colorectal cancer patient comprising screening a cell or tissue sample isolated from the patient for the genotype at codon 118 of the ERCC1 gene, wherein the genotype (C/C) selects the human patient for treatment with said therapy. Claim 12 has been canceled without prejudice or disclaimer. Claims 4 and new claims 16 -19 further define claim 1 by selecting the fluoropyrimidine to be 5-Fluorouracil and/or the platinum drug to be oxaliplatin, or further defining the therapy to include radiation therapy. New claim 19 recites a method for determining whether a human metastatic colorectal cancer patient is likely to experience longer survival following treatment with said therapy comprising the administration of a fluoropyrimidine and a platinum drug by screening a cell or tissue sample isolated from the patient for a genotype at codon 118 of the ERCC1 gene, wherein the presence of the genotype (C/C) at codon 118 of the ERCC1 gene indicates. New claims 21-26, are dependent on claim 19 and further define the claim by selecting the fluoropyrimidine to be 5-Fluorouracil and/or the platinum drug to be oxaliplatin, further defining the therapy to include radiation therapy. New claim 27 recites a method for treating a human metastatic colorectal cancer patient by administering an effective amount of a therapy comprising a fluoropyrimidine and a platinum drug to a patient selected for the therapy based on the possession of the genotype (C/C) at codon 118 of the ERCC1 gene. Claims 28-32, are dependent on claim 27 and further define the claim by selecting the fluoropyrimidine to be 5-Fluorouracil and/or the platinum drug to be oxaliplatin, or further defining the therapy to include radiation therapy.

Each claim presented for examination has the element, either explicitly or implicitly through dependence directly or indirectly on an independent claim to require selecting a therapy, identifying a metastatic colorectal cancer patient likely to or less

likely to exhibit longer survival time or treating a patient based on correlating the presence of the genotype (C/C) of the ERCC1 gene for selection of a therapy with a fluoropyrimidine and a platinum drug. This claim element is absent from the teachings of Yu et al. Furthermore, whereas Yu et al. teaches that the polymorphism is present in ovarian cancer, without regard to its relationship to any treatment. In order to anticipate a claimed invention, the reference must teach each and every element of the claim and enable the practice of the claimed subject matter. Yu et al. fails to meet this standard as it fails to correlate the presence of the genotype (C/C) at codon 118 of the ERCC1 gene with treatment for metastatic colorectal cancers now claimed by Applicants. For these reasons, the amended and new claims are not anticipated by the disclosure of Yu et al. Reconsideration and withdrawal of the rejection is therefore respectfully requested.

III. CONCLUSION

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check or credit card payment form being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741.

If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicants hereby petition for such extension under 37 C.F.R. §1.136 and authorize payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date May 14, 2008 By Antoinette F Konski

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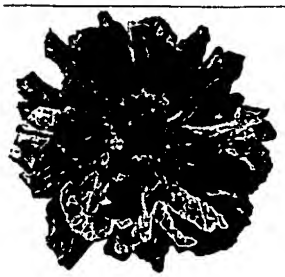
Exhibit A



The Oncologist®

The Use of Thymidylate Synthase Inhibitors in the Treatment of Advanced Colorectal Cancer: Current Status

D. Papamichael

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The Use of Thymidylate Synthase Inhibitors in the Treatment of Advanced Colorectal Cancer: Current Status

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Key Words. *Thymidylate synthase · 5-Fluorouracil · S-1 · UFT · Capecitabine · Raltitrexed · MTA*

ABSTRACT

The combination of 5-fluorouracil (5-FU) and leucovorin has been the unofficial "standard" therapy for patients with colorectal cancer for over a decade. Recently, however, a number of new agents targeted against the enzyme thymidylate synthase have been synthesized and are in various stages of development. The currently available thymidylate synthase inhibitors are discussed.

Enormous efforts have been made over the years to improve the efficacy of 5-FU, the most popular of these agents. Biochemical modulation by leucovorin has been the most successful so far. Continuous infusion schedules also appear to be advantageous over bolus administration. However, marked intra- and interpatient variability, combined with nonlinear elimination kinetics and erratic oral bioavailability are relative limitations to further development of 5-FU. New oral 5-FU prodrugs such as UFT, S-1, and Capecitabine may help to overcome some of these difficulties. Eniluracil, a potent inhibitor of the enzyme dihydropyrimidine dehydrogenase, may also help by overcoming potential 5- FU resistance mechanisms, in addition to increasing its bioavailability. Of the antifolate-based inhibitors, Tomudex is in the most advanced stage of development. Similar efficacy with 5-FU and a convenient schedule may suggest a role in future combination regimens.

It is quite likely that even the most optimal thymidylate synthase inhibition will have limitations in terms of clinical efficacy. Novel combinations of 5-FU or its analogs with agents that have different mechanisms of action (e.g., oxaliplatin, irinotecan) could provide important new opportunities for improving the outlook of patients with colorectal cancer. *The Oncologist 1999;4:478-487*

INTRODUCTION

Colorectal cancer (CRC) is the fourth most common malignancy globally and the second leading cause of cancer deaths in Western countries, with approximately 300,000 new cases per annum diagnosed in the USA and Europe. The three major types of therapy in CRC are surgery, chemotherapy, and radiation therapy, each one of them applied differently, depending on whether the aim of treatment is curative or palliative. Approximately 50% of patients will ultimately die of locally advanced or metastatic disease [1]. Only a minority of patients with metastases qualify for surgical resection. Consequently, the most widely used approach for this group is systemic or locoregional chemotherapy combined with, where appropriate, palliative radiotherapy. Randomized trials have shown that chemotherapy improves both survival and quality of life (QOL) in advanced CRC [2-5]. Treatment with 5-Fluorouracil (5-FU) and calcium leucovorin (LV) has been the "standard" therapy for patients with CRC for over a decade. Recently however, a number of new agents targeted against thymidylate synthase (TS) have been synthesized and are in various stages of development. The purpose of this article is to review the currently available TS inhibitors used in the treatment of advanced CRC.

THYMIDYLATE SYNTHASE INHIBITION

An important feature of nucleotide metabolism is the duplication of pathways; inhibition of any one enzyme can be circumvented by one or more alternative routes. One notable exception to this is TS, a "bottleneck" enzyme which provides the only means of adding a methyl group to the 5-position of

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the pyrimidine ring in the de novo synthesis of thymidine. Thymidylate synthase is also the one enzyme in the nucleotide synthesis pathway which, instead of metabolizing the 5-FU derivative to its natural substrate, is instead inhibited by it.

Since thymidine is the only nucleotide precursor specific to DNA, TS is an obvious target for cytotoxic agents. The enzyme's activity is a two-stage process. First, deoxyuridine monophosphate (dUMP) binds to a receptor site; this induces a configurational change which opens an adjacent binding site for N-5,10-methylene-tetrahydrofolate (CH2FH4). The folate's one carbon group is then transferred to the uridine ring, yielding deoxythymidine monophosphate (dTMP) and dihydrofolate [6, 7]. dTMP is subsequently phosphorylated by a kinase to dTDP and dTTP, one of the bases for DNA synthesis (Fig. 1).

It has long been known that fluorodeoxyuridine monophosphate (FdUMP), a 5-FU metabolite, potently inhibits TS, and that this is one of the main mechanisms underlying 5-FU action [7]. It binds at the same site and with the same affinity as dUMP (KS 1-2 × 10^{-6} M), but unlike hydrogen, the fluorine atom at the 5′ position cannot be displaced. Subsequently, FdUMP and the reduced folate become covalently bound with TS, to form a ternary complex (Fig. 2), where a cysteine thiol of TS is attached to the 6′ position of FdUMP, with the one-carbon group of the folate adjacent to F at the 5′ position (Fig. 3).

5-FU and Its Prodrugs

Since its introduction over 40 years ago by *Heidelberger* [8], 5-FU has been the drug of choice for systemic therapy in CRC, although it can be argued that the optimum treatment strategy for its use has not been fully established. Enormous efforts have been made to improve the efficacy of 5-FU, either by changing its biochemical modulation and/or the method of administration. These efforts have been inspired by new insights into the biochemical pharmacology of 5-FU and the identification of agents that effectively enhance the antitumor activity of 5-FU in preclinical models. The three potential ways of modifying its activity are: A) through manipulation of dose or schedule; B) by the addition of other agents to modulate the activity of 5-FU and thus

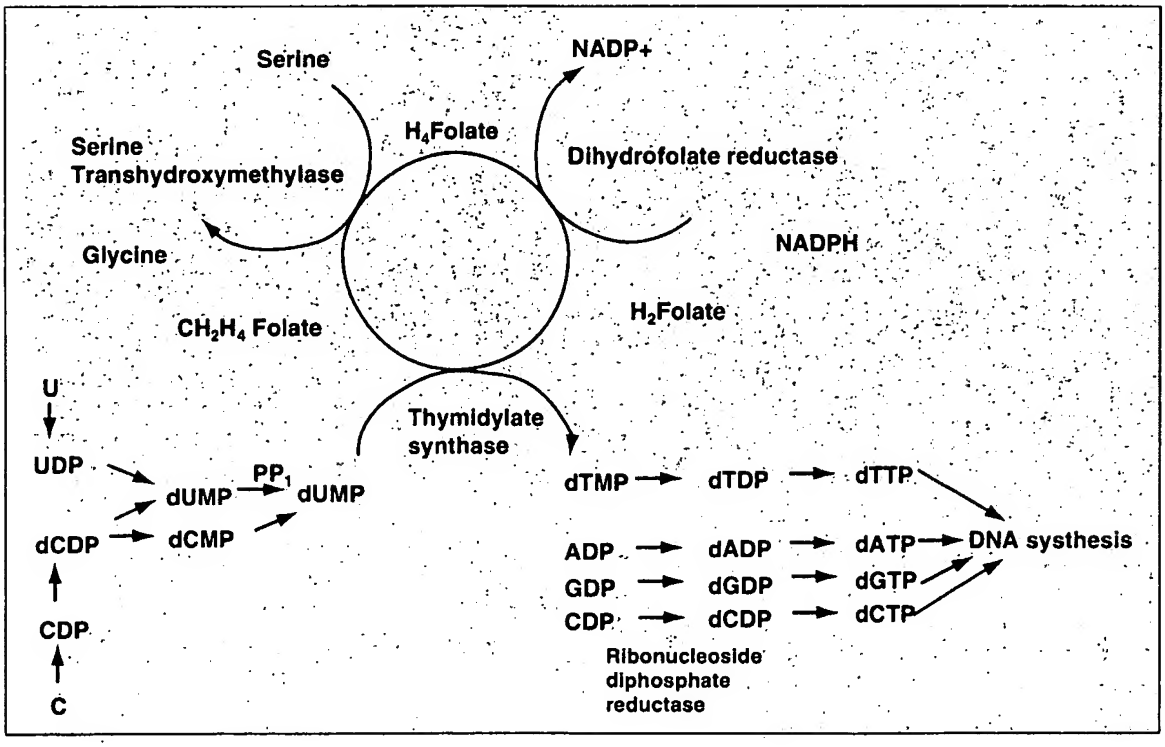

Figure 1. In the thymidylate synthesis cycle, dietary folate is reduced to dihydrofolate, which is further reduced by the enzyme dihydrofolate reductase to tetrahydrofolate, using dihydronicotinomide adenine dinucleotide phosphate as a hydrogen source. Tetrahydrofolate is then converted to methylenetetrahydrofolate by the enzyme serine transhydroxymethylase, which uses vitamin B6 as a cofactor. The methylene-group-carrying cofactor, methylene tetrahydrofolate, then provides both a methylene group and reducing activity, to convert dUMP to dTMP by the action of the enzyme thymidylate synthase (U = uridine; C = cytidine; PP1 = pala pyrazofurin; A = adenosine; G = guanosine; d = deoxy-; DP = diphosphate; MP = monophosphate; TP = triphosphate).

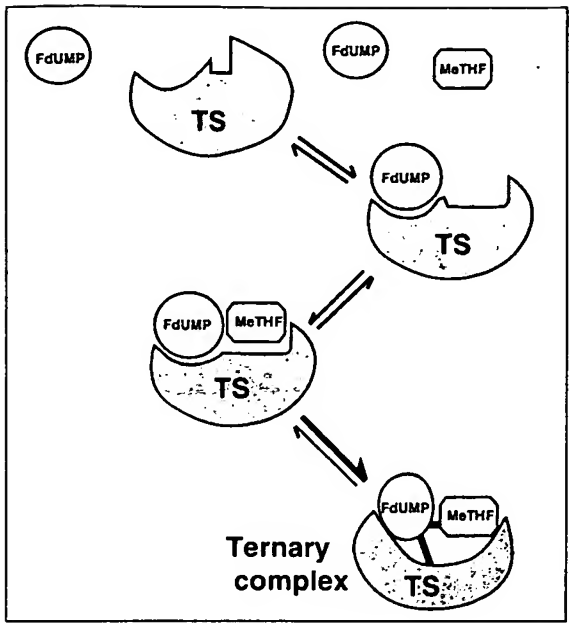

Figure 2. FdUMP/folate binding to TS.

producing metabolic or functional interactions, and C) by the use of analogs/prodrugs.

5-FU Scheduling

5-FU is characterized by marked schedule dependency in both the quality and quantity of its effects. This has been demonstrated in numerous studies, in vitro, in animal models, and in clinical practice, where bolus and infusional regimens have been compared and contrasted in terms of their activity and toxicity profiles [9]. Bolus 5-FU is more likely to exert its effect through an action on RNA, whereas infusional 5-FU is more likely to work through TS. Differences in the two schedules can be seen in diverse settings; e.g., an improvement in survival was reported in an adjuvant randomized chemoradiotherapy rectal cancer trial, when a protracted infusional 5-FU regimen was used, as compared with bolus [10]. Unmodulated bolus 5-FU appears to have a threshold dose intensity (500-600 mg/m²/wk) [11]. A similar dose-response relationship also appears to exist with 5-FU continuous-infusion (CI) regimens [12]. The threshold dose

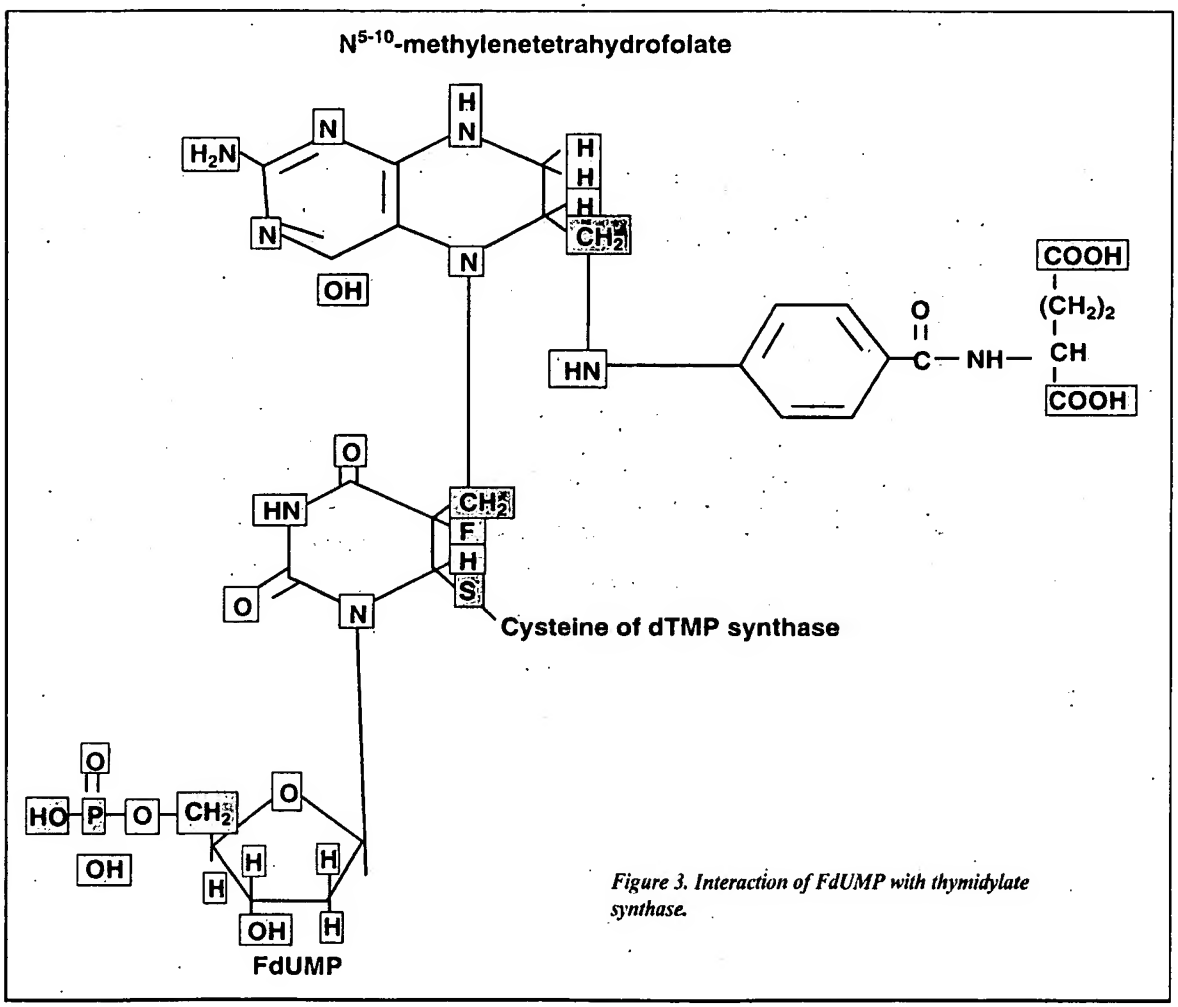

Figure 3. Interaction of FdUMP with thymidylate synthase.

for clinical activity for CI regimens is likely to be in the range of 1,500 mg/m²/wk; on the other hand, there is probably no therapeutic gain in giving more than 2,600 mg/m²/wk [13, 14]. It should be noted that dose intensity achieved with CI regimens is three to four times higher than that of bolus 5-FU. In 1998, a meta-analysis of all randomized trials comparing infusional and bolus administration of 5-FU was published [15]. A database of just over 1,200 patients was covered. Tumor response was significantly higher in the CI arm (22% versus 14%, $p = 0.002$). Furthermore, although median survival times were close, overall survival was significantly higher for patients on infusional 5-FU (hazard ratio = 0.88, $p = 0.04$).

5-FU Modulation

The use of modulating agents to enhance the activity of 5-FU is currently widely employed. To date, the most successful and commonly used biochemical modulator of 5-FU is LV. Many clinical trials have now been performed with the combination, including several phase III trials comparing 5-FU plus LV with 5-FU alone. The meta-analysis of 1,381 patients in nine randomized clinical trials confirmed the advantage of treatment with the combination in terms of objective response [16]. The response rate of the combination, as compared with 5-FU alone, was 23% versus 11% ($p < 10^{-7}$). This benefit was documented in trials involving weekly and daily schedules of administration with both high-dose LV (>200 mg/m²/day) and low-dose LV (<25 mg/m²/day). The meta-analysis, however, did not reveal an advantage in terms of survival.

One disappointing aspect of the clinical experience with 5-FU and LV is that toxicity to normal tissues is increased, necessitating a reduction in the dose of 5-FU administered. This is contrary to the hope and expectation that normal tissues, having adequate baseline folate pools, would be unaffected by the addition of LV. The clinical toxicity profile of 5-FU modulated by LV seems to be more dependent on the schedule of 5-FU rather than on the dose of LV [17]. Study of the 5-FU/LV combination continues both in the advanced as well as the adjuvant settings, so that questions relating to the optimum use of LV (e.g., high versus low dose) can be resolved.

The use of methotrexate (MTX) as a modulating agent has also been investigated, although its use has not been so widespread. This is despite a meta-analysis showing a survival advantage for the use of MTX in combination over 5-FU alone [18]. The use of other agents such as interferon (IFN) and hydroxyurea has so far been less promising. In the case of interferon, a number of randomized phase III clinical trials conducted to assess the addition of IFN to a number of different "popular" 5-FU/LV regimens, proved very disappointing [19-22]. In all of the above studies, it became evident that while toxicity was increased by the addition of IFN, there was no enhanced efficacy. The initial phase II data, on which the enthusiasm for the use of IFN was based [23], were therefore not confirmed.

The clinical pharmacology of 5-FU is characterized by marked intra- and interpatient variability, non-linear elimination kinetics, and erratic oral availability. As the administered dose increases, 5-FU displays saturable pharmacokinetics, i.e., the plasma half-life increases, plasma clearance decreases, hepatic extraction decreases, and the area under the concentration-time curve (AUC) increases disproportionately. As a result, the plasma concentration achieved with any given dose is dependent both on the dose administered and the rate of the infusion. Whether 5-FU plasma concentrations can be used to individualize dosing remains controversial [24]. In view of the above limitations, a number of agents were developed in an effort to overcome some of these problems.

Eniluracil

Eniluracil is a potent inactivator of dihydropyrimidine dehydrogenase (DPD). The recent recognition of the clinical importance of DPD—the first enzyme in a degradation pathway that rapidly catabolizes more than 80% of orally administered 5-FU—has led to new potential strategies for improving the efficacy and safety of 5-FU administration. The importance of DPD on 5-FU pharmacology has been further emphasized by results of studies suggesting the influence of DPD on pharmacokinetics, bioavailability, toxicity, and efficacy of 5-FU. DPD follows a circadian pattern in animals and humans [25]. Also, there is DPD interpatient variability (enzyme activity), taking a gaussian pattern, in some instances responsible for severe toxicity in patients with low DPD who are exposed to 5-FU for the first time [26].

In animal models, pretreatment with eniluracil significantly increased the bioavailability and reduced the variability of oral 5-FU, giving a pharmacokinetic pattern similar to that produced when the drug is administered i.v. [27, 28]. It has also been demonstrated in vitro that DPD activity was an independent factor significantly related to 5-FU sensitivity. It is possible, therefore, that the inhibition of DPD by eniluracil in colorectal tumors may eliminate a potential mechanism of 5-FU resistance [29].

In a phase I trial of oral eniluracil plus i.v. 5-FU with or without oral leucovorin, the dose-limiting toxicities (DLT) were neutropenia and diarrhea [30]. Clinical responses in CRC patients were noted. Pharmacokinetic data showed that eniluracil decreases the clearance of 5-FU and prolongs its half-life. Another phase I trial assessed several different regimens of oral eniluracil plus oral 5-FU [31]. It was noted from that study that the bioavailability of oral 5-FU was greatly increased, and that there was a marked decrease in interpatient variability. Renal excretion appeared to be the principal

mechanism for 5-FU elimination. The DLT for the combination was neutropenia, and this precluded escalation of 5-FU to doses >25mg/m²/day for five days in combination with eniluracil given at 3.7 mg/m²/day on days 1-7 every four weeks. With this oral 5-FU dose, the systemic exposure achieved was comparable to that observed using 5-FU doses of 1,000 mg/m²/day for five days as a CI. *Mani et al.* reported preliminary results from a phase II study in CRC, where response rates of 25% and 29% were observed for 1.0 and 1.15 mg/m² b.d. respectively combined with eniluracil [32].

On the basis of the above promising results, two large phase III trials (FUMA308 and FUMB3002) are currently under way in the USA and Europe. The toxicity pattern in these trials will be of great interest. If side effects such as hand-foot syndrome, cardiotoxicity, and neurotoxicity are related to 5-FU catabolism, one might expect these to be less pronounced in patients receiving a DPD inhibitor.

5-FU Analogs/Prodrugs

5'-deoxy-5-fluorouridine

A number of other fluoropyrimidines have been synthesized, most of which act as prodrugs for 5-FU. For example, the 5'-deoxynucleoside, 5'-deoxy-5-fluorouridine (doxifluridine) must have its ribosyl group removed by the enzyme uridine phosphorylase to produce 5-FU [33]. This enzyme is reported to be more active in some tumor cells than in normal tissues, resulting in an improved therapeutic ratio in tumor-bearing mice [34]; however, very high activity is found in normal human liver, casting doubt on doxifluridine's claimed sensitivity [35]. Unfortunately, clinical trials have been marked by severe neurotoxicity, and there is as yet no evidence that it gives any clinical benefit over 5-FU, except that it may be used orally.

UFT

Another prodrug, ftorafur (1-tetrahydrofuranyl-5-fluorouracil) is metabolized to 5-FU either by hepatic P-450 microsomal enzymes or by ubiquitous cytosolic enzymes [36]. It has the advantage of 100% bioavailability. Its activity is improved by the coadministration of uracil in a 4:1 molar ratio ("UFT"), which blocks its degradation by DPD and gives more prolonged concentration of 5-FU in tumor tissues [37, 38].

The toxicities observed with UFT, which is orally administered, are typical of the fluoropyrimidines. Phase II studies with oral UFT in a variety of cancers showed comparable activity to single-agent i.v. 5-FU [39]. Randomized studies to date, comparing either 5-FU/cisplatin versus ftorafur/cisplatin in patients with head and neck cancer [40] or UFT and 5-FU in combination with cyclophosphamide and doxorubicin in advanced breast cancer [41] showed no significant differences in terms of activity or toxicity. Furthermore, in a study comparing the pharmacokinetic profile of oral UFT to that of protracted i.v. 5-FU, it was concluded that the AUC of 5-FU generated was equal in both treatments [42]. In addition, UFT peak and disappearance curves followed the same pattern as for 5-FU. Already, phase III trials in advanced CRC are well under way, comparing UFT in combination with LV versus "standard" 5-FU/LV regimens. Early results from two phase III trials, giving a combined experience from just under 1,200 patients, reported similar efficacy for the two treatment arms. However, the combination of UFT/LV was thought to be safer in terms of toxicity and a more convenient oral alternative to 5-FU [43, 44].

The potential for combining UFT with other agents may have a wider application [45]. Also, the ease of oral administration of UFT eliminates the need for long-term indwelling catheters and their accompanying complications.

Capecitabine

Capecitabine (Xeloda), is a new orally administered, tumor-activated, and tumor-selective fluoropyrimidine carbamate. After absorption through the intestinal mucosa as the intact molecule, due to its carbamate structure and thus potentially causing less diarrhea, it is converted to 5'-deoxy-5-fluorouridine by a sequential triple enzyme pathway. The last tumor-selective enzyme reaction is mediated by the tumor-associated angiogenic factor thymidine phosphorylase, when it is further metabolized to 5-FU. Theoretically, therefore, it has two major advantages, which may translate into an improved therapeutic index: first, enhanced drug concentration at the cancer site and therefore greater anti-tumor activity, and second, reduced drug levels in non-tumor tissues, with a consequent reduction in systemic toxicity.

Preclinical data have suggested an improved efficacy profile over 5-FU and oral UFT, with tumor selectivity for Capecitabine's activation [46]. In xenograft models, concentrations of 5-FU were found to be higher in tumor than in plasma or healthy tissue (127-fold higher than in plasma, and 22-fold higher than in muscle). In contrast, selective distribution of 5-FU was not observed following 5-FU administration.

Side effects in early clinical trials were similar to those observed with infusional 5-FU, including hand-foot syndrome. Results from a randomized phase II clinical trial carried out with three dosage schedules were somewhat less encouraging, at least in terms of response rates [47]. Two phase III trials enrolling patients with advanced colorectal carcinoma are comparing an intermittent capecitabine schedule with i.v. 5FU/LV ("Mayo"), in terms of efficacy as well as quality of life and pharmacoeconomic resource parameters. Early results,

reported so far only in abstract form, show higher response rates (26.6% versus 17.9%, $p = 0.013$ [48], and 23.2% versus 15.5%, $p = 0.02$ [49]) and a more favorable toxicity profile than modulated bolus 5-FU [48, 49]. The same regimen of capecitabine is also being evaluated in a large-scale adjuvant trial, which is expected to recruit approximately 1,700 Duke's C colon cancer patients (X-ACT study).

S-1(BMS-247616)

Another potentially very interesting orally bioavailable 5-FU prodrug is S-1. It consists of tegafur and two modulators: a 5-chloro-2,4-dihydroxypyridine (CDHP—an inactivator of DPD—200 times more potent than uracil), and potassium oxonate (inactivator of normal gastrointestinal tissue phosphoribosyl pyrophosphate transferase, thereby reducing diarrhea), causing decreased drug incorporation into cellular RNA. As with UFT, the aim of S-1 is to mimic 5-FU prolonged CI. Preclinical as well as early clinical data point to encouraging activity particularly in advanced gastric cancer (49% complete and partial response rate) with a mild toxicity profile [50], and possible synergistic activity when combined with cisplatin [51]. Preliminary data from a phase I study show effective inhibition of DPD, with diarrhea as the main toxicity. In this study, the maximum tolerated dose was 45 mg/m^2 given in two daily doses for four weeks, followed by a one-week rest. In a phase II study that used a schedule of 100 mg/m^2/day for four weeks, the response rate was 17% in 30 patients with CRC [51].

Direct TS Inhibitors

Currently, four folate-based agents that have been designed to interact with the folate-binding site of TS by using binding side-structure analysis, are either available as licensed products or in clinical trials at various stages. These are: raltitrexed (Tomudex); nolatrexed, LY231514, a multitargeted antifolate (MTA), and ZD9331. As well as displaying interesting clinical activity, these new drugs also illustrate the principles of rational drug design being used to explore and exploit the features of folate metabolism.

Although 5-FU inhibits TS, it also has major effects in other pathways, as well—in particular, RNA metabolism. Specific TS blockade is therefore not achieved. Also, as TS inhibition causes an increase in intracellular dUMP pools, dUMP will eventually compete with 5-FU for binding to TS. In contrast, folate-based inhibitors have distinct advantages; they can inhibit TS without significantly influencing other folate-dependent enzymes. Additionally, dUMP enhances the binding of folate analogs to TS.

Raltitrexed (TOM)

Raltitrexed, a water-soluble antifolate agent, is a specific inhibitor for TS. Being a classical antifolate, it possesses a terminal glutamate residue which is converted to a polyglutamate form in the cell. Polyglutamates tend to be significantly more potent than monoglutamates as enzyme inhibitors and are also retained in the cell for long periods. Potential causes of resistance to the drug would arise from the fact that it requires a specific transport protein to cross the cell membrane, as well as the fact that it functions as a prodrug for its polyglutamate form [52]. Pharmacokinetic studies indicate a triphasic elimination with a wide range in the mean terminal half-life (8.2-105 h). There is a linear relationship between the dose and both the AUC and the maximum concentration, although there is no clear association between these parameters and either clinical response or toxicity.

In the clinical setting, a reasonable side-effect profile from phase I [53] and a promising overall response rate (26%) in patients with advanced colorectal cancer in phase II studies, led to the initiation of three randomized phase III trials [54-56]. The European trial [54] compared TOM with 5-FU/LV administered using the "Mayo" schedule. In 439 patients, this study showed no significant difference between the two treatments in terms of response rate (19% versus 17%), median survival (10.3 months), or time to progression (4.7 versus 3.6 months). The North American trial made the same comparison in 459 patients [55]. The response rate for the two treatments at 12 months' follow-up was comparable (14% and 15% respectively), although the median survival time was significantly shorter for the TOM treatment group (9.7 versus 12.7 months). The international trial that compared TOM with 5-FU/LV ("Machover schedule") in 495 patients reported comparable response rates (19% versus 18% respectively) and median survival times (10.7 versus 11.8 months) for the two treatments [56]. Raltitrexed appears to have an advantage in terms of less myelotoxicity and mucositis, but a disadvantage in terms of transaminitis (reversible), nausea and sometimes prolonged asthenia, as compared with 5-FU. The use of alternative doses and schedules (apart from the 3 mg/m^2 bolus every three weeks) has not been widely explored, although the 4 mg/m^2 bolus every three weeks schedule was found to be too toxic in one of the phase III trials reported above [55].

A Pan-European Intergroup Trial (PETACC-1) has been launched to compare 5-FU/LV ("Mayo") versus TOM in an adjuvant setting for stage Duke's C colon cancer. In the meantime, the Medical Research Council of the United Kingdom is conducting a study comparing TOM with two different 5-FU schedules ("de Gramont"—a bi-monthly bolus and CI 5-FU/LV 48-h regimen [57] and Protracted Venous Infusion-PVI 5-FU [58]) in the advanced disease setting. The recruitment of just over 900 patients, which is now complete, will hopefully provide firmer conclusions in relation to TOM's place in the management of advanced CRC. However, preliminary results from the above study

suggest that TOM may be inferior to infusional 5-FU in terms of treatment-related deaths, progression-free survival, and QOL [59].

Nolatrexed (Thymitaq, AG337)

Nolatrexed is a water-soluble lipophilic inhibitor of TS which does not have glutamate side chains and can enter the cell by passive diffusion. This makes it different from other antifolates such as TOM or MTA (see below). It has a short plasma half-life, and since it cannot form polyglutamates and thus be retained in cells, this necessitates a prolonged (5-day) infusion. Its spectrum of toxicities is more similar to 5-FU than TOM, with myelosuppression and mucosal toxicity being dose-limiting [60]. Limited activity in colorectal and pancreatic cancer has been observed [61, 62], deserving further evaluation. More recently, while in phase II evaluation, nolatrexed was withdrawn from further clinical study; the reasons for this are not as yet clear.

LY231514—MTA

MTA has multiple targets in the folate pathway. It causes inhibition of a range of enzymes involved in folate metabolism, the most important being TS, dihydrofolate reductase (DHFR), human monofunctional glycinamide ribonucleotide formyltransferase (hGARFT), and aminoimidazole carboxamide ribonucleotide formyltransferase (AICARFT). MTA is transported across the cell membrane using the reduced folate carrier system (like TOM) and has low affinity to folate receptors, as well as being an excellent substrate for the enzyme FPGS.

In phase I studies its DLT were neutropenia and thrombocytopenia for all schedules tested [63]. Other side effects included mucositis, rashes, and transient elevation of transaminases. The three-weekly schedule (minor responses seen in six patients with colorectal cancer) was chosen for phase II evaluation in breast and colorectal cancer. Early results indicate encouraging activity in colorectal cancer, with responses in the range of 15%-17% in a total of just over 70 patients [64, 65].

ZD9331

This molecule is of particular interest because of its unique preclinical profile. It is a potent TS inhibitor, while neither being lipophilic nor polyglutamated [66]. It is currently undergoing phase I studies, displaying an extremely long half-life in the range of 70 to 120 hours. No phase II data are as yet available.

Conclusion

Until recently, 5-FU was the only available drug with moderate but nevertheless consistent activity in CRC. Biochemical modulation and new administration schedules have occasionally led to improved response rates and a more favorable side-effect profile, but have not really had a significant impact on survival. Several new drugs have now become available and are in various stages of development. The 5-FU prodrugs may be used either A) to allow a more convenient schedule (e.g., oral therapy), or B) to exploit a tumor-specific prodrug-activating enzyme. Of these, UFT, capecitabine, and S-1 have shown interesting response rates in phase II studies, and early results from ongoing phase III studies (for the first two agents) are encouraging. Their potential advantages in terms of ease of administration and possible improved therapeutic index may have a significant impact on their future use. At the very least, the above agents offer an alternative simplified strategy for achieving protracted exposure to fluoropyrimidine chemotherapy, eliminating the need for indwelling catheters and special infuser pumps. This would make outpatient treatment much more feasible and might have a significant impact on the patient's QOL as well as healthcare costs. The biochemical modulator ethinyluracil, also allows the oral administration of 5-FU and has the potential to overcome one of 5-FU's resistance mechanisms; however, results of phase III clinical trials are not yet available. Among the new antifolates, raltitrexed is in the most advanced stage of development. It does not appear to be more effective than 5-FU/LV as first-line treatment, although its convenient schedule may suggest a possible role in future combination treatments.

All of the agents described above have TS as their primary target. It is quite likely that even the most optimal TS inhibition will have its limitations in terms of clinical efficacy. A combination of TS inhibitors with agents that have a different mechanism of action seems a logical approach. Two drugs that may well fulfill this role are irinotecan (CPT-11)—a topoisomerase-1 inhibitor [67], and oxaliplatin—a new DACH platinum compound with a favorable side-effect profile [68]. Both agents have shown activity in first- and second-line treatment either by themselves or in combination with various schedules of 5-FU/LV [69, 70]. Preliminary phase III data are already available, indicating the superiority of both agents in combination with 5-FU over 5-FU alone [71, 72]. It is quite clear that we are entering a new era in the treatment of colorectal cancer, with new agents likely to change the so far standard 5-FU/LV combinations that have dominated the landscape of CRC treatment for the past decade. The use of these new cytotoxic agents together with the advent of novel biologic therapies will hopefully translate into a significant and meaningful survival advantage for this group of patients.

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Exhibit B

ADIS DRUG EVALUATION

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Oxaliplatin

A Review of its Use in the Management of Metastatic Colorectal Cancer

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Data Selection

Sources: Medical literature published in any language since 1966 on oxaliplatin, identified using AdisBase (a proprietary database of Adis International, Auckland, New Zealand), Medline and EMBASE. Additional references were identified from the reference lists of published articles. Bibliographical information, including contributory unpublished data, was also requested from the company developing the drug.

Search strategy: AdisBase search terms were 'oxaliplatin', 'ACT-078', and 'L-OHP'. Medline and EMBASE search terms were 'oxaliplatin', 'ACT-078' and 'oxalatoplatin' and 'eloxatin'. Searches were last updated April 1999.

Selection: Studies in patients with metastatic colorectal cancer who received oxaliplatin. Inclusion of studies was based mainly on the methods section of the trials. When available, large, well controlled trials with appropriate statistical methodology were preferred. Relevant pharmacodynamic and pharmacokinetic data are also included.

Index terms: Oxaliplatin, colorectal cancer, pharmacodynamics, pharmacokinetics, therapeutic use.

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Summary

Abstract

Oxaliplatin is a cytotoxic agent which, like other platinum compounds, acts primarily by causing inter- and intra-strand cross-links in DNA, thereby inhibiting DNA synthesis. Oxaliplatin has a bulky carrier ligand which is thought to enhance cytotoxicity and abolish cross-resistance between oxaliplatin and other platinum compounds.

Phase II and III clinical trials have found oxaliplatin combined with fluorouracil/calcium folinate (leucovorin/folinic acid) to be an effective first- and second-line treatment for patients with metastatic colorectal cancer. First-line triple therapy with oxaliplatin and fluorouracil/calcium folinate achieved significantly higher response rates than fluorouracil/calcium folinate alone in 2 phase III studies (objective response rates 59 *vs* 23% and 50.7 *vs* 22.3%). In addition, median progression-free survival was longer with triple therapy in both studies (8.9 *vs* 5.2 and 8.75 *vs* 6.0 months). However, there was no significant difference in median duration of survival between treatment groups, although this may be a consequence of the subsequent use of oxaliplatin and/or surgery in patients who relapsed during therapy with fluorouracil/calcium folinate alone.

About 30 to 45% of patients (whose disease progressed during or after fluorouracil-based therapy) responded to second-line combination therapy with oxaliplatin and fluorouracil/calcium folinate. Median progression-free survival ranged from 7 to 10 months and the median duration of survival from 10 to 17 months.

Objective responses were achieved in 20 and 24% of patients in 2 small trials of first-line oxaliplatin monotherapy and in about 10% of patients given the drug as a second-line option.

Peripheral sensory neuropathy is the dose-limiting toxicity associated with oxaliplatin. Severe neurotoxicity has been estimated to occur in 10% of patients after 6 treatment cycles and in 50% after 9 cycles of an oxaliplatin dosage of 130 mg/m^2 once every 3 weeks. It is cumulative, but reversible on discontinuation of therapy. Nausea, vomiting and diarrhoea are common, but are usually mild to moderate. Myelosuppression is also observed, but is usually mild.

Conclusion: oxaliplatin is a promising treatment option for patients with metastatic colorectal cancer. It appears to be particularly advantageous (in terms of response rate and duration of progression-free survival) when used in combination with fluorouracil/calcium folinate as both a first- and second-line option, although preliminary studies have failed to show any survival advantage over fluorouracil/calcium folinate alone. Promising results have been found in studies of the drug as monotherapy, and oxaliplatin may also prove useful in the neoadjuvant setting in patients with unresectable liver metastases; however, data are limited at present.

1. Introduction

Colorectal cancer is one of the leading causes of cancer death in the industrialised world. It has been estimated to claim the lives of at least 18 000 patients in the UK and 55 000 in the US each year, with similar mortality rates in other industrialised countries.[1,2] Colorectal cancer is highly metastatic, with approximately 50% of patients eventually dying from disease progression. Thus, the long term outcome for patients diagnosed with metastatic disease is poor, as most patients are resistant to current treatment options and the most common site of metastases is the liver. Consequently, treatment is largely palliative, the aim being to improve quality of life.

Fluorouracil has been the most consistently used chemotherapy for metastatic colorectal cancer in the last 40 years. Objective response rates to monotherapy with this agent are typically about 10 to 20%, with a median survival duration of about 10 months.[3] Approaches to further improve response rates have included modifications of the fluorouracil dosage schedule, the addition of biochemical modifiers and use of fluorouracil in combination with other chemotherapeutic agents. The addition of biochemical modulating agents such as calcium folinate (leucovorin/folinic acid) may increase the response rate by up to 2-fold.[3-8]

Second-line treatment options in patients who do not respond to, or who progress during or after, fluorouracil-based therapy are few. Until recently, retreatment with fluorouracil, often in combination with other drugs, has been the most common option (response rates ranging from 0 to 17%[9-13]).

Thus, attempts have been made to identify new agents or treatment strategies which can achieve higher response rates and have a greater effect on survival than current fluorouracil-based treatment regimens. Some of these approaches are summarised in table I. Agents showing at least similar clinical efficacy to fluorouracil and/or efficacy in patients refractory to, or relapsing after, fluorouracil-based therapy include oxaliplatin (the focus of this review), irinotecan (a semisynthetic derivative of the topoisomerase I inhibitor camptothecin)[14] and raltitrexed (a quinazoline-based folate analogue that inhibits thymidylate synthase).[15] Combination of these agents with fluorouracil-based therapy may further improve response rates.[16]

Oxaliplatin, a diaminocyclohexane (DACH) platinum compound (fig. 1), is the first platinum-based drug to show efficacy in colorectal cancer. Oxaliplatin has also shown efficacy in the treatment of patients with advanced ovarian cancer,[17-20] and preliminary results indicate that the drug may also be useful in the treatment of breast[21,22] and non-small cell lung cancer[23] and non-Hodgkins lymphoma;[24] however, this review focuses solely on its use in metastatic colorectal cancer.

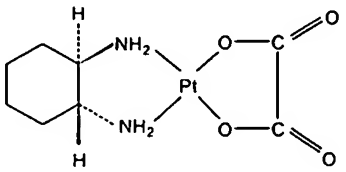
2. Pharmacodynamic Properties

2.1 Mechanisms of Action and Resistance

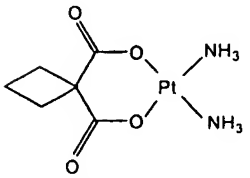
Although the precise mechanism is unclear, platinum compounds are thought to exert their cytotoxic action in a similar manner to alkylating agents by causing inter- and intrastrand cross-links in DNA.[25]

Table I. Summary of strategies under investigation for the treatment of metastatic colorectal cancer[16]

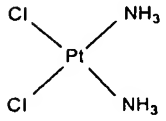
Approaches to enhance fluorouracil/calcium folinate (folinic acid/leucovorin) activity (e.g. using the antifolate trimetrexate, chronomodulated drug delivery)
Novel delivery of fluorouracil precursor agents (e.g. administration of oral fluorouracil prodrugs)
New thymidylate synthase inhibitors (e.g. raltitrexed)
Platinum analogues (e.g. oxaliplatin)
Topoisomerase I inhibitors (e.g. irinotecan)
Targets for specific proteins or pathways important for the growth, survival or metastasis of colorectal cancer cells (e.g. ras inhibitors, inhibitors of protein kinase C, cyclin-dependent kinases and growth factor receptors)
Biological response modifiers (e.g. recombinant interferon-α, recombinant interleukin-2 and monoclonal antibodies targeted against a variety of tumour cell antigens)
Gene therapy (e.g. administration of the p53 gene via an adenoviral vector)



Oxaliplatin



Carboplatin



Cisplatin

Fig. 1. Chemical structures of oxaliplatin, carboplatin and cisplatin.

Intrastrand cross-links result from the formation of adducts between activated platinum complex and areas of specific base sequences. The degree of cytotoxicity is related to DNA binding, and the major cytotoxic lesion is thought to be an intrastrand bifunctional adduct that cross-links 2 adjacent guanines or 2 adjacent guanine-adenine bases.[25,26] Interstrand cross-links make up less than 5% of the total platinum-DNA adducts, but have also been implicated in cytotoxic effects resulting from interference with DNA replication and transcription processes.[25] Thus, platinum compounds bind to nucleophilic sites on DNA and inhibit DNA synthesis.

The type and site of DNA lesion caused by oxaliplatin appears to be the same as with other platinum compounds,[27,28] but DACH-platinum DNA adducts formed by oxaliplatin are generally associated with greater cytotoxicity and inhibition of DNA synthesis than the *cis*-diamine-platinum DNA adducts formed by cisplatin or carboplatin.[28-31] The bulky DACH carrier ligand of oxaliplatin is thought to contribute to this enhanced activity as well as to the lack of cross-resistance between oxaliplatin and cisplatin.

Multiple mechanisms are involved in the development of tumour cell resistance to platinum compounds. These include reduced formation of cytotoxic platinum-DNA adducts (e.g. via decreased drug accumulation or increased inactivation) and increased repair of, or tolerance to, platinum-DNA damage, as well as defects in DNA mismatch repair and increased replicative bypass (table II).[25,32-34] However, defects in mismatch repair and increased replicative bypass appear to be the only mechanisms that discriminate between cisplatin and DACH-platinum compounds such as oxaliplatin.[32]

2.2 *In Vitro* Activity

In *in vitro* studies, the antiproliferative activity of oxaliplatin was equivalent to or greater than that of cisplatin against murine leukaemia (L1210, P388) and a variety of human cancer cell lines, including HT29 and HCT116 (DNA mismatch repair-deficient) colon carcinoma (fig. 2), breast (MCF-7), ovarian (A2780), endometrial (HEC59) and neuroblastoma cell lines.[29,35-39]

Although DACH-platinum compounds such as oxaliplatin are not effective in all cisplatin-resistant cell lines,[40,41] some studies have found oxaliplatin to have markedly greater cytotoxicity than either cisplatin or carboplatin against various drug-resistant

Table II. Mechanisms of cancer cell resistance to platinum compounds;[25,32] mechanisms shown to discriminate between oxaliplatin and cisplatin/carboplatin are indicated by †

Decreased cellular drug accumulation (i.e. reduced passive diffusion and/or carrier-mediated transport)
Drug inactivation by intracellular metallothionine (sequestration) or glutathione (conjugation)
Enhanced excision repair of platinum-DNA adducts
Enhanced tolerance of platinum-DNA adducts by alterations of the various components involved in programmed cell death (apoptosis)
†Defects in DNA mismatch repair
†Increased replicative bypass

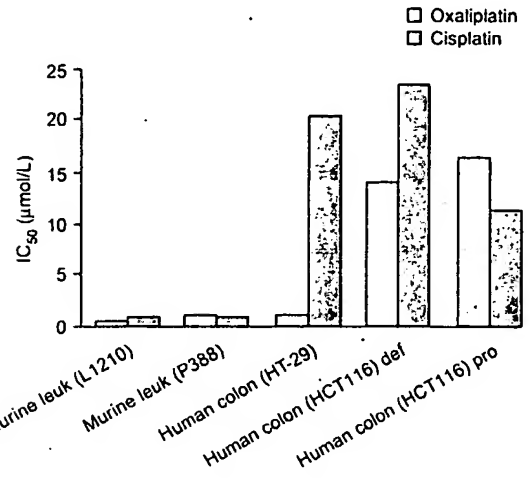


Fig. 2. Median or mean concentrations of oxaliplatin and cisplatin inhibiting proliferation of various murine and human tumour cell lines by 50% (IC_{50}).[35-37] **def** = DNA mismatch repair-deficient; **leuk** = leukaemia; **pro** = DNA mismatch repair-proficient.

human cancer cell lines (e.g. ovarian, non-small cell lung cancer, and epithelial cancers).[30,42,43] One noteworthy study evaluated the cytotoxicity of platinum compounds against 2 cisplatin-resistant cell lines (and their cisplatin-sensitive counterparts) from the US National Cancer Institute human cancer cell line panel.[30] The median concentration required to inhibit proliferation by 50% (IC_{50}) was 0.12 μmol/L for oxaliplatin against cisplatin-sensitive ovarian cancer A2780 (1A9), which increased 4.7-fold against cisplatin-resistant ovarian cancer A2780-E80. For cisplatin, the IC_{50} value was 0.21 μmol/L against the sensitive cell line but this was increased 92-fold against the resistant cell line; respective values for carboplatin were 0.35 μmol/L and 64-fold resistance. A similar pattern was also observed with a cisplatin-resistant epithelial HeLa subclone [KB CP(20)] which demonstrated 2.7-fold resistance against oxaliplatin, 78-fold resistance against cisplatin and 57-fold resistance against carboplatin compared with its cisplatin-sensitive counterpart (KB 3-1). In a separate analysis, oxaliplatin was significantly more cytotoxic against nonseminomatous germ cell cancer cell lines with either acquired (H12DDP clone) or intrinsic (1777NRp C1-A clone) intermediate levels of resistance to cisplatin, although the cytotoxic activity of oxaliplatin was not significantly different from that of cisplatin against cell lines which were cisplatin-sensitive or those with a high level (10- to 20-fold) of cisplatin resistance.[42]

The cytotoxic activity of oxaliplatin has also been evaluated using clonogenic assays in which the drug's *in vitro* potency was assessed against a variety of human tumour colony-forming units isolated from patients.[44] Oxaliplatin had marked cytotoxic activity against colon, non-small cell lung, gastric and ovarian cancer cells, and responses were dependent on length of exposure and oxaliplatin concentration. Significant cytotoxic activity was demonstrated with oxaliplatin against clones resistant to various conventional chemotherapeutic agents.

A number of *in vitro* studies have also shown that some oxaliplatin-based combinations have additive or synergistic activity against certain cell lines (reviewed by Raymond et al.[32]). For colon cancer cell lines specifically, such effects were demonstrated with oxaliplatin plus fluorouracil (against cell lines HT29, HT29-5-FU, CaCo2), gemcitabine (against cell lines HCT116, Colo 320 DM), topoisomerase I inhibitors irinotecan (CPT-11) or SN-38 (the active metabolite of irinotecan) [against cell line HT29] or the thymidylate synthase inhibitor AG337 (against cell line HT29).[45-47] However, antagonistic or less than additive effects have been demonstrated in some *in vitro* studies evaluating oxaliplatin-based combinations, including simultaneous exposure of HCT116 and HCT8 human colon cancer cells to oxaliplatin plus SN38 (the active metabolite of irinotecan).[48]

2.3 *In Vivo* Activity

The antitumour activity of oxaliplatin has been compared with that of cisplatin in a number of murine models of haematological[49-51] and solid tumours.[51,52] In general, the antineoplastic activity of oxaliplatin was greater than that of cisplatin against L1210 leukaemia, fibrosarcoma M5067 and MA16-C mammary carcinoma, and comparable to that of cisplatin against P388 leukaemia, L40 AkR leukae-

mia, B16 melanoma and colon 38 carcinoma. In mice grafted with solid tumours, oxaliplatin (dosage range used in the various studies: 5 to 12.5 mg/kg) was associated with approximately 1.4- to 3.5-fold improvements in survival compared with control groups of mice (presumably receiving placebo vehicle), and survival was increased by a factor of about 1.8 to 3.1 versus controls in murine models of haematological tumours. However, in mice with cisplatin-resistant L1210 leukaemia, oxaliplatin 6.25 mg/kg prolonged survival by more than 7 times compared with the same dose of cisplatin.[51] Oxaliplatin 5 mg/kg was also associated with a cure rate of >50% in mice with LGC lymphoma, whereas cisplatin 5 mg/kg was inactive.[49] It is also noteworthy that, at least in this series of experiments, neither oxaliplatin nor cisplatin demonstrated activity against grafts of glioma 26, B16 melanoma, MA16-C mammary adenocarcinoma or Lewis lung carcinoma.[49]

A number of *in vivo* models have shown that some oxaliplatin-based combinations have additive or synergistic activity (reviewed by Raymond et al.[32]). Such combinations include the following: oxaliplatin plus fluorouracil against the human colon carcinoma HT29 xenograft, GR mouse mammary tumours and L1210 leukaemia;[45,46,52] oxaliplatin plus either SN38, CPT-11 or AG337 against GR mouse mammary tumours;,[45,46] oxaliplatin combined with both paclitaxel and tirapazamine against the human lung cancer MV-522 xenograft (reported by Raymond et al.[32]); and oxaliplatin plus either cisplatin or carboplatin against L1210 leukaemia.[53]

3. Pharmacokinetic Properties

The pharmacokinetic profile of oxaliplatin has been investigated in patients with various malignancies who received an intravenous infusion of oxaliplatin alone or in combination with fluorouracil/calcium folinate.

3.1 Disposition

Approximately 2 hours after intravenous administration, oxaliplatin is no longer detectable in plasma ultrafiltrate; in its place are several platinum biotransformation products. These include (*trans*-1,2-DACH) dichloroplatinum, methionine (*trans*-1,2-DACH) platinum and glutathione (*trans*-1,2-DACH) platinum.[54]

In patients with metastatic colorectal cancer, a maximum total plasma platinum concentration (C_{max}) of 3.2 mg/L was achieved 2 hours after completion of a short term oxaliplatin infusion (130 mg/m^2 over 2 hours).[55] By day 8, the total plasma platinum concentration had declined to 0.44 mg/L.[55] The plasma concentration curve for ultrafiltrable platinum resembled that of total platinum (peak 0.45 mg/L at 2 hours followed by a rapid decline).

During administration of 7 cycles of chemotherapy comprising oxaliplatin 130 mg/m^2 once every 3 weeks and fluorouracil/calcium folinate once weekly, accumulation of platinum in plasma (including ultrafiltrable platinum) was minimal.[55] However, after 3 treatment cycles, there was significant accumulation of platinum in red blood cells (91% increase in erythrocyte platinum concentration on day 22 of third cycle *vs* day 22 of first cycle; $p < 0.01$).[55] In an *in vitro* study,[37] 37% of available platinum accumulated in erythrocytes within 2 hours when oxaliplatin 5 to 20 mg/L was incubated with whole blood. Further data from this study indicated that intraerythrocyte platinum is not exchangeable into the plasma and does not act as a drug reservoir.[37]

The effect of peak infusion time on plasma platinum levels was investigated in 36 patients with metastatic colorectal cancer treated with chronomodulated oxaliplatin 25 mg/m^2/day, fluorouracil 800 mg/m^2/day and calcium folinate 150 mg/m^2/day for 4 days. Total platinum C_{max} was dependent upon the peak time of the oxaliplatin infusion and was significantly lower after delivery of oxaliplatin at 0100 hours than at 0700 or 1600 hours (0.7, 1.2 and 1.0 mg/L, respectively; $p < 0.001$).[56]

Oxaliplatin-derived platinum is extensively bound to human plasma proteins. In patients with cancer, the proportion of protein bound platinum was 70% at 2 hours and increased to 95% 5 days after infusion of oxaliplatin 130 mg/m^2 over 2 hours.[57]

3.2 Metabolism and Elimination

The platinum-containing biotransformation products of oxaliplatin are excreted principally in the urine. After intravenous administration of oxaliplatin 130 mg/m^2 to patients with cancer, approximately 50% of the platinum dose was excreted in the urine within 72 hours and this increased slightly to 57% by day 11.[57] Elimination of platinum in the faeces is minimal (approximately 5% after 11 days). Oxaliplatin is undetectable in the urine within 2 hours of intravenous administration,[54,55] but total platinum is still detectable in significant amounts in plasma after 22 days.[55]

Although the systemic clearance of ultrafiltrable platinum was significantly reduced (≈40%) in patients with renal impairment [creatinine clearance (CL_{CR}) <3.6 L/h] compared with healthy volunteers (15.1 *vs* 26.5 L/h; $p < 0.05$), total platinum systemic clearance (protein and nonprotein bound platinum) was similar in the 2 patient groups (1.9 *vs* 2.4 L/h).[54]

The mean total platinum terminal elimination half-life ($t_{½\beta}$) in plasma was 9 days after administration of oxaliplatin 130 mg/m^2 over 2 hours, and $t_{½\beta}$ of ultrafiltrable platinum was 7.15 days.[55] Elimination of platinum from erythrocytes is extremely prolonged ($t_{½\beta}$ = 48 days).[55]

3.3 Drug Interactions

Two briefly reported studies have investigated the effect of oxaliplatin on the pharmacokinetic profile of fluorouracil and have shown contrasting results. In 1 study,[58] concomitant administration of oxaliplatin (dose not specified) significantly decreased the area under the plasma concentration-time curve (AUC) of fluorouracil by ≈30% from monotherapy values and significantly increased linear elimination of the drug (≈70%).[58] In the second study,[59] plasma levels of fluorouracil were significantly increased by ≈30% from monotherapy values during concomitant administration of oxaliplatin 130 mg/m^2.

In another study,[60] the AUC of irinotecan 200 mg/m^2 after coadministration with oxaliplatin 85 mg/m^2 was similar to that reported with irinotecan monotherapy 350 mg/m^2 (≈26 mg/L · h).[60]

4. Clinical Efficacy

Oxaliplatin has been trialled as both first- and second-line therapy in patients with metastatic colorectal cancer. Most data pertain to its use in combination with fluorouracil-based therapy; however, phase II trials have studied oxaliplatin monotherapy, and a recent trial has investigated combination therapy with oxaliplatin and the topoisomerase I inhibitor irinotecan. Several regimens of oxaliplatin have been used (table III), which varied in dosage, duration of infusion and frequency of administration. Both standard (fixed rate) and chronomodulated infusion schedules have been investigated. Chronomodulation is based on circadian rhythm, with peak delivery of fluorouracil and calcium folinate at 0400 hours and/or peak delivery of oxaliplatin at 1600 hours.

Table III provides a summary of the largest trials of oxaliplatin. Most of these have been published in full; however, only interim results are available from 2 large multicentre randomised phase III studies[61-64] comparing first-line fluorouracil/calcium folinate therapy with or without oxaliplatin.

Patients enrolled in clinical trials of oxaliplatin were aged 18 to 75 years and were generally required to have biopsy-proven adenocarcinoma, measurable recurrent or metastatic disease, a WHO performance status ≤2 and a life expectancy >1 to 3 months. In studies of oxaliplatin as first-line therapy, previous chemotherapy or radiation for metastatic disease was not allowed. However, patients who had received prior adjuvant or neoadjuvant chemotherapy and/or radiotherapy were eligible for the study if they had a disease-free period of ≥6 months after treatment completion.

The primary efficacy end-point was the objective response rate, i.e. complete plus partial response rate. Secondary end-points included the duration of response, time to disease progression (or treatment failure), duration of progression-free survival and median duration of survival. The incidence of disease stabilisation was also reported by most investigators.

Tumour responses were evaluated according to WHO criteria.[70]

Table III. Efficacy of oxaliplatin (OXA) as first-line therapy in patients with metastatic colorectal cancer

Reference	No. of patients (pts)	Treatment regimen (mg/m^2/day) [frequency]	Objective response rate (% pts) [CR + PR]	Disease stabilisation (% pts)	Median time to disease progression (mo)	Median progression-free survival (mo)	Median duration of survival (mo)
Combination therapy (OXA + 5-FU/FA) versus 5-FU/FA							
Figer et al.,[61] de Gramont et al.[62] (mc, r)	420 total	OXA 85 2h inf d1 + 5-FU 400 B then 5-FU 600/FA 200 CI d1-2 [q2wk]	50.7*** [NR]	NR	NR	8.75***	17.25
		5-FU 400 B then 600/FA 200 CI d1-2 [q2wk]	22.3 [NR]	NR	NR	6.0	16
Giacchetti et al.[63-65] (mc, r)	100	OXA 125 6h inf d1 + 5-FU 700/FA 300 CM d1-5	59[a]*** [NR]	NR	NR	8.9[a]	17.6
	100	5-FU 700/FA 300 CM d1-5	23[a] [NR]	NR	NR	5.2[a]	19.4
Chronomodulated versus continuous OXA infusion + 5-FU/FA							
Levi et al.[66] (mc, r)	47 (6% had AC)	OXA 20-25 CM + 5-FU 600-700/FA 300 CM d1-5 [q3wk]	53* [6 + 47]	36	NR	11	19*
	45 (11% had AC)	OXA 20-25 CI + 5-FU 600-700/ FA 300 CI d1-5 [q3wk]	32 [4 + 28]	46	NR	8	14.9
Levi et al.[67] (mc, r)	93 (14% had AC)	OXA 20-25 CM + 5-FU 600-700/ FA 300 CM d1-d5 [q3wk]	50** [5 + 45]	NR	6.4[b]*	9.8	15.9
	93 (13% had AC)	OXA 20-25 CI + 5-FU 600-700/ FA 300 CI d1-d5 [q3wk]	29 [3 + 26]	NR	4.9[b]	7.9	16.9
Oxaliplatin monotherapy							
Becouarn et al.[68]	38	OXA 130 2h inf d1 [q3wk]	24 [0 + 24]	41	7.2	4.2	13.2
Diaz-Rubio et al.[69]	25	OXA 130 2h inf d1 [q3wk]	20[c] [4 + 16]	32	6	4.0	14.5

a According to investigators. An independent expert committee reported an objective response rate of 53 *vs* 16% and median progression-free survival of 7.9 *vs* 4.3 months for oxaliplatin combination therapy versus fluorouracil/calcium folinate therapy, respectively.

b Median time to treatment failure.

c According to investigators. An independent expert committee reported an objective response rate of 12%.

5-FU = fluorouracil; **AC** = adjuvant chemotherapy; **B** = bolus; **CI** = continuous infusion; **CM** = chronomodulated delivery rate (12-hour infusion of oxaliplatin with peak delivery at 1600 hours followed by 12-hour infusion of fluorouracil/calcium folinate with peak delivery at 0400 hours); **CR** = complete response (defined in section 4); **d** = days; **FA** = calcium folinate; **Inf** = intravenous infusion; **IRIN** = irinotecan; **mc** = multicentre; **mo** = months; **NR** = not reported; **PR** = partial response; **pts** = patients; **q** = every; **r** = randomised; **wk** = week; * $p < 0.05$; ** $p < 0.005$; *** $p < 0.001$ *vs* comparator.

- complete response: total disappearance of all measurable and assessable lesions documented at 2 observations 4 weeks apart with no new lesions appearing
- partial response: ≥50% decrease in tumour area (sum of the products of the greatest length and the maximum perpendicular width of all measurable lesions) with no new lesions appearing and no increase in size in any previous lesions
- stable disease: <25% increase in indicator lesions with no new lesions appearing
- progressive disease: ≥25% increase in one or more lesions or the appearance of new lesions.

Treatment was continued until disease progression or the occurrence of intolerable toxicity.

4.1 First-Line Therapy

4.1.1 Combination Therapy

Interim results from 2 large multicentre randomised phase III studies indicate that combination therapy with oxaliplatin and fluorouracil/calcium

folinate achieves a significantly higher objective response rate than fluorouracil/calcium folinate alone. The objective response rate (according to investigators) was 59% with combination therapy *vs* 23% with fluorouracil/calcium folinate in one study (53 *vs* 16% according to experts),[63-65] and 50.7 *vs* 22.3% in the other ($p < 0.001$ in both studies).[61,62] These studies used different drug dosages and infusion schedules (for details see table III). The median progression-free survival time in patients given combination oxaliplatin/fluorouracil/calcium folinate therapy was longer than that in patients given only fluorouracil/calcium folinate in the 2 phase III studies described above (8.9 *vs* 5.2 months[65] and 8.75 *vs* 6.0 months[61]). The difference was statistically significant in the latter study.[61]

Median survival did not differ significantly between treatment groups in either study (17.6 *vs* 19.4 months[63,64] and 17.25 *vs* 16 months[61]) [table III]. The long survival in both treatment groups may be accounted for by the fact that oxaliplatin therapy and/or surgery was allowed after disease progression in patients initially randomised to receive only fluorouracil/calcium folinate.[63] Indeed, in the study by Giacchetti et al.,[63] 57 of 100 patients were given oxaliplatin as second line therapy, and of these, 42 (72%) showed a further response (10 had partial responses and 32 had stable disease).

In an analysis of prognostic factors for overall survival, Figer et al.[61] found that, although no significant survival benefit was observed with oxaliplatin in the randomised trial,[71] the addition of oxaliplatin to fluorouracil/calcium folinate therapy was a significant prognostic factor for longer survival.

Chronomodulated versus Fixed Rate Drug Infusion

In a study comparing infusion regimens of combination therapy, response rates were significantly higher in patients given a sequential chronomodulated treatment regimen (12-hour infusion of oxaliplatin with peak delivery at 1600 hours followed by 12-hour infusion of fluorouracil/calcium folinate with peak delivery at 0400 hours) than in those given standard fixed rate infusion.[66] The response rate in patients given fluorouracil 600 to 700 mg/m^2/day, calcium folinate 300 mg/m^2/day and oxaliplatin 20 to 25 mg/m^2/day for 5 days once every 3 weeks at a variable (chronomodulated) or fixed infusion rate was 53 *vs* 32% ($p < 0.05$). This result was also found in a larger subsequent study by the same investigators in which response rates with chronomodulated and fixed rate infusions were 51 *vs* 29% ($p < 0.005$).[67] Median progression-free survival appeared longer in patients given chronomodulated drug delivery than in those given a fixed rate infusion (11 *vs* 8 months[66] and 9.8 *vs* 7.9 months[67]). These differences did not differ significantly; however the time to treatment failure was significantly longer with chronomodulated drug delivery in one of these studies (6.4 *vs* 4.9 months; $p = 0.006$).[67] Chronomodulated delivery of fluorouracil/calcium folinate and oxaliplatin was associated with a significantly longer median duration of survival than fixed rate infusion (19 *vs* 14.9 months; $p < 0.05$) in one study;[66] however, in a subsequent larger study conducted by the same investigators the median duration of survival did not differ significantly between treatment groups.[67]

4.1.2 Monotherapy

Limited data are available on the use of oxaliplatin as first-line monotherapy. Objective response rates in 2 small phase II studies in which the drug was administered at a dosage of 130 mg/m^2 infused over 2 hours once every 3 weeks were 20 and 24% (table III).[68,69] All other end-points were similar for the 2 studies; median time to disease progression 7.2 and 6 months, median progression-free survival 4.2 and 4 months and median survival duration 13.2 and 14.5 months.[68,69]

4.2 Second-Line Therapy

4.2.1 Combination Therapy

Combination therapy with oxaliplatin and fluorouracil/calcium folinate achieved objective response rates typically between 30 and 45% in most phase II trials of second-line therapy (table IV).

Median progression-free survival ranged from 7 to 10 months and the median duration of survival was between 10 and 17 months.[72-74,77-79] These studies used a variety of treatment regimens which

Table IV. Efficacy of oxaliplatin (OXA) as second-line therapy in patients with metastatic colorectal cancer

Reference	No. of patients (previous treatment for advanced disease)	Treatment regimen (mg/m^2/day) [frequency]	Objective response rate (% patients) [CR + PR]	Disease stabilisation (% patients)	Median time to disease progression (mo)	Median progression-free survival (mo)	Median duration of survival (mo)
Combination therapy (noncomparative studies)							
Berthault-Cvitkovic et al.[72]	37 (fluorouracil-based)	OXA 25 CM + 5-FU 700-1100/FA 300 CM d1-d4 [q2wk]	43 [3 + 40]	54	NR	9.3	16.9
Gerard et al.[73]	36 (fluorouracil-based)	OXA 130 2h inf d1 q3wk + FA 500 1h inf/5-FU 2600 CI d1, d8, d22, d29, d43	28 [0 + 28]	17	10	NR	10
Levi et al.[74]	93 (43% fluorouracil-based)	OXA 25 CM + 5-FU 700/FA 300 CM d1-5 [q3wk]	57 [6 + 51]	36	NR	10	15
Scheithauer et al.[75]	36 (fluorouracil-based)	OXA 85 2h inf d1, d15 + IRIN 80 30 min inf d1, d8, d15 [qwk]	42 [6 + 36]	36	7.5[a]	NR	NR[b]
Combination therapy (comparative studies)							
de Braud et al.[76]	12 (fluorouracil-based)	OXA 130 2h inf d1 + 5-FU 200-300 CI or 375 B /FA 100 d1-5 [q3wk]	33[0 + 33]	33	4	NR	NR
de Gramont et al.,[77,78] Andre et al.[79]	13 (fluorouracil-based)	OXA 130 2h inf [q4wk] + FA 500 2h inf + 5-FU 1500-2000 22h inf d1-2 [q2wk]	31 [0 + 31]	38	NR	NR	11
	60 (fluorouracil-based)	OXA 100 + FA 500 2h inf + 5-FU 1500-2000 22h inf d1-2 [q2wk]	37 [2 + 35]	35	NR	NR	15
	46 (fluorouracil-based)	OXA 100 + FA 500 2h inf + 5-FU 1500-2000 22h inf d1-2 [q2wk]	46 [2 + 44]	NR	NR	7	17
	40 (fluorouracil-based)	OXA 85 + FA 500 2h inf + 5-FU 1500-2000 22h inf d1-2 [q2wk]	16 [0 + 16]	53	NR	NR	10

Table IV. Contd

Reference	No. of patients (previous treatment for advanced disease)	Treatment regimen (mg/m²/day) [frequency]	Objective response rate (% patients) [CR + PR]	Disease stabilisation (% patients)	Median time to disease progression (mo)	Median progression-free survival (mo)	Median duration of survival (mo)
	30 (fluorouracil-based)	OXA 85 + FA 500 2h inf + 5-FU 1500-2000 22h inf d1-2 [q2wk]	20 [NR]	50	NR	6.5	14.25
Monotherapy							
de Braud et al.[76]	12 (fluorouracil-based)	OXA 130 2h inf d1 [q3wk]	0	33	2	NR	NR
Levi et al.[80]	29 (86% fluorouracil-based)	OXA 30-40 CM d1-5 [q3wk]	10 [0 + 10]	24	NR	5	10
Machover et al.[81]	55 (fluorouracil-based	OXA 130 2h inf d1 [q3wk]	11 [0 + 11]	42	6	NR	8.2
Machover et al.[81]	51 (fluorouracil-based)	OXA 130 2h inf d1 [q3wk]	10 [0 + 10]	31	4.5	NR	NR

a Median time to treatment failure.

b Median survival duration not yet reached. After a median follow-up of 14 months, 19 (53%) patients are still alive.

5-FU = fluorouracil; **B** = bolus; **CI** = continuous infusion; **CM** = chronomodulated delivery rate (12-hour infusion of oxaliplatin with peak delivery at 1600 hours followed by 12-hour infusion of fluorouracil/calcium folinate with peak delivery at 0400 hours); **CR** = complete response (defined in section 4); **d** = days; **FA** = calcium folinate; **h** = hour; **Inf** = intravenous infusion; **IRIN** = irinotecan; **mc** = multicentre; **mo** = months; **NR** = not reported; **PR** = partial response; **pts** = patients; **q** = every; **r** = randomised; **wk** = weeks; * $p < 0.05$; ** $p < 0.005$; *** $p < 0.001$ *vs* comparator.

differed in dosage, duration of infusion and frequency of administration of oxaliplatin and of fluorouracil-based chemotherapy (standard or high dosage).

In dose-finding studies of combination therapy comprising fixed-rate infusions of high dose fluorouracil (1500 to 2000 mg/m²/day) and calcium folinate (500 mg/m²/day) administered every 2 weeks plus oxaliplatin (doses 85, 100 or 130 mg/m²), an oxaliplatin dosage of 100 mg/m² once every 2 weeks produced the best response rate (37% of 60 patients had an objective response in one study and 46% of 46 patients in another).[77-79]

Results from a recent small phase II trial (n = 36 patients previously treated with fluorouracil/calcium folinate) indicate that combination therapy with oxaliplatin and irinotecan is a promising alternative for second-line therapy of metastatic colorectal cancer.[75] The overall response rate was 42% (6% complete and 36% partial responses), with a further 36% of patients showing disease stabilisation.

4.2.2 Monotherapy

Approximately 10% of patients with tumours resistant to fluorouracil had an objective response with second-line oxaliplatin monotherapy, administered either as a fixed dose (130 mg/m² by 2-hour infusion every 21 days)[81] or as a chronomodulated 5-day continuous infusion (30 to 40 mg/m²/day with peak delivery at 1600 hours)[80] in phase II studies (table IV). However, it is noteworthy that in 1 small study of 12 patients, none responded to oxaliplatin monotherapy. The median time to disease progression ranged from 2 to 6 months and the median survival duration was 8.2 and 10 months with second-line oxaliplatin monotherapy in 2 studies.[80,81]

4.3 Use in Patients with Unresectable Liver Metastases

Bismuth et al.[82,83] investigated the effect of oxaliplatin therapy on liver metastases in patients with initially unresectable disease. Response rates

varied markedly; however, triple chronomodulated therapy with oxaliplatin and fluorouracil/calcium folinate shrank tumours to a surgically manageable size in 16% of patients (53 of 330 patients entered into clinical trials to investigate chronomodulated infusion). 37 patients underwent major hepatectomy and 16 had minor resection. The cumulative 3- and 5-year survival rates were 54 and 40%, respectively. The investigators concluded that this compared well with survival obtained with primary liver resection (40% at 5 years).

5. Tolerability

In clinical trials of oxaliplatin, toxicity was recorded after each chemotherapy course and, in most studies, graded according to WHO[70] or National Cancer Institute common toxicity criteria (NCI-CTC)[84] for haematological, skin, mucosal and hair toxicity. Nausea, vomiting, diarrhoea and peripheral sensitive neuropathy were assessed according to specifically modified WHO grading systems (see section 4).

The dose-limiting toxicity in patients receiving oxaliplatin is peripheral sensory neuropathy. This is cumulative, but reversible in most patients a few months after stopping treatment. It is associated with sensory ataxia and dysesthaesia of the limbs, mouth, throat and larynx. Symptoms may be exacerbated by exposure to cold.[85,86] Grade 3 neurotoxicity occurred in 13% of 39 patients given oxaliplatin as first-line therapy at the recommended dosage of 130 mg/m^2 once every 3 weeks.[68] When oxaliplatin (85 mg/m^2 once every 2 weeks) was combined with fluorouracil/calcium folinate, the incidence of grade 3 neurotoxicity was 15% in a phase III study in which the drugs were delivered by continuous infusion (rather than chronomodulated infusion).[87] An analysis of data from 682 patients enrolled in 9 clinical trials found that the risk of developing severe neurotoxicity was 10% after 6 cycles (dosage 130 mg/m^2) and 50% after 9 cycles of treatment.[86] In this analysis, the incidence of grade 3 to 4 neurotoxicity was 3% with oxaliplatin monotherapy and 19% with combination therapy (fig. 3).

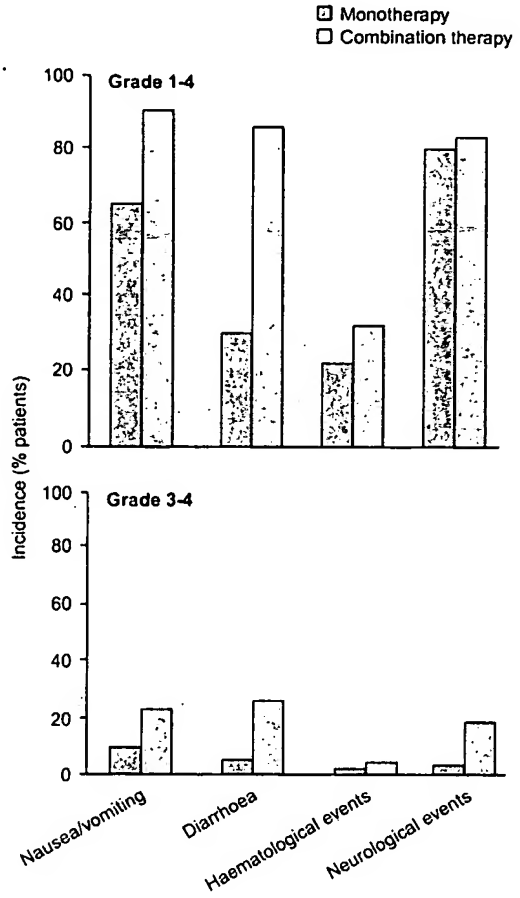


Fig. 3. Tolerability of oxaliplatin monotherapy and combination therapy with oxaliplatin/fluorouracil/calcium folinate in patients with metastatic colorectal cancer.[86] WHO toxicity grades 1 to 4 and 3 to 4 are shown. Data are from an analysis of 682 patients enrolled in 9 clinical trials in which the dosage regimens varied (see table III).

A sporadic laryngopharyngeal dysesthaesia, thought to result from decreased sensitivity of the larynx and pharynx, may be observed after drug infusion (in about 1 to 2% of patients). The resultant feeling of difficulty in breathing or swallowing is distressing to the patient, but symptoms resolve within hours of onset.[85,88]

Nausea, vomiting and diarrhoea are common adverse events; however, they are generally mild to moderate (fig. 3). Oxaliplatin has not been associated with renal or auditory toxicity.[85]

Severe anaphylactic reactions have been observed rarely.[89]

The relative incidences of adverse events in patients given either oxaliplatin or fluorouracil/calcium folinate or combination therapy with all 3 agents are summarised in figures 3 and 4. Although no statistically significant difference in overall tolerability between monotherapy and combination therapy was reported (fig. 3), the incidences of severe (WHO grade 3 to 4) diarrhoea, nausea/vomiting and neurological events were higher in patients given combination therapy.[71,86] Grade 3 or 4 diarrhoea, vomiting, stomatitis and neutropenia also appeared more common in patients given triple therapy with oxaliplatin and fluorouracil/calcium folinate than in those given fluorouracil/calcium folinate alone; however, statistical significance was not reported (fig. 4).

The tolerability of combination therapy may be improved by sequential chronomodulated delivery of the drugs. In a randomised comparative study of dosage regimens, the incidence of stomatitis was significantly higher in patients given a fixed rate infusion than in those given a chronomodulated one (76 *vs* 14%; p = 0.0001).[66,67,74] Peripheral sensory neuropathy was the cumulative dose-limiting toxicity with both dosage regimens; however, it was more frequent in patients given the fixed rate infusion (31 *vs* 16%). The incidence of diarrhoea (35 *vs* 29%) and vomiting (25 *vs* 24%) was similar with both administration regimens. The rate of withdrawal because of intolerable adverse events was significantly lower in patients given the chronomodulated regimen (28 *vs* 51%; p = 0.002), as was the rate of hospital admissions for grade 4 events (10 *vs* 31%; p = 0.001).

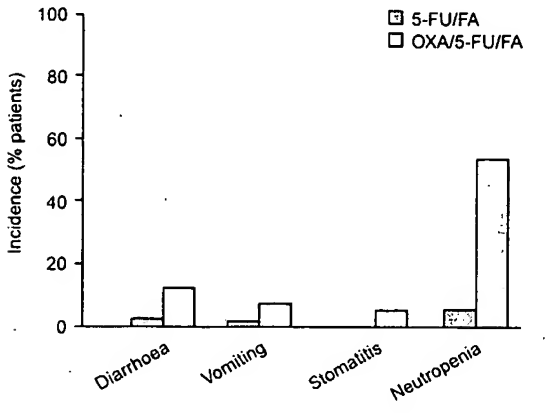


Fig. 4. Incidence of severe adverse events (WHO grade 3-4) in 200 patients with metastatic colorectal cancer given fluorouracil (400 mg/m^2 bolus then 600 mg/m^2)/calcium folinate (200 mg/m^2) [5-FU/FA] for 2 days alone or with oxaliplatin (OXA) [85 mg/m^2] once every 2 weeks.[71] Neutropenia was asymptomatic. Statistical significance was not reported.

6. Dosage and Administration

Oxaliplatin in combination with fluoropyrimidines is recommended for the treatment of metastatic colorectal cancer. The drug may be given as monotherapy to patients in whom fluoropyrimidine therapy is contraindicated.[88]

The recommended dosage of oxaliplatin for combination with fluoropyrimidines is 85 mg/m^2 once every 2 weeks or 130 mg/m^2 once every 3 weeks. The dose should be administered as at 2- to 6-hour intravenous infusion and given before fluoropyrimidine therapy.

As monotherapy, the recommended dose is 130 mg/m^2 once every 3 weeks.

Contraindications to the use of oxaliplatin include known allergy to the drug, pregnancy and breastfeeding. Caution is advised in patients with a history of allergic reaction to platinum compounds. Patients should be monitored for neurological toxicity; if acute laryngopharyngeal dysesthaesia develops following the 2-hour infusion, the next infusion should be given over 6 hours.[88]

7. Place of Oxaliplatin in the Management of Metastatic Colorectal Cancer

Treatment options for metastatic colorectal cancer are few, with fluorouracil-based therapy being the mainstay of treatment at present. However, objective response rates are low (typically 10 to 20% with fluorouracil-based treatment), and therapy has little influence on survival (median duration 10 months).[3] In addition, most tumours eventually become resistant to fluorouracil.

Combination therapy with oxaliplatin and fluorouracil/calcium folinate appears to have a synergistic effect, typically achieving objective responses in ≥50% of patients when used as first-line and 30 to 45% as second-line therapy. Preliminary reports of phase III studies have shown this triple therapy regimen to be superior to fluorouracil/calcium folinate alone as first-line therapy, in terms of response rate and median progression-free survival. However, available data have failed to show any survival advantage of adding oxaliplatin to fluorouracil-based therapy. As mentioned previously, this may be a consequence of the fact that second-line oxaliplatin and/or surgery was allowed in patients who relapsed during first-line therapy with fluorouracil/calcium folinate.

The efficacy of oxaliplatin monotherapy relative to that of other treatment options remains unclear. However, studies in small numbers of patients indicate that the drug may be a useful first-line option in some patients (objective response rates were 20 and 24% and median survival 13.2 and 14.5 months in 2 phase II studies). These response rates compare well with those achieved with the newer agent raltitrexed, which has also been investigated as a first-line treatment option (response rates 14 to 26% and median survival duration 9.7 to 11.2 months).[15]

The dose-limiting toxicity in patients receiving oxaliplatin therapy is peripheral sensory neuropathy. This is cumulative, dose-related and reversible. It is characterised by dysesthaesia and/or distal paraesthesia, often exacerbated by cold. Oxaliplatin has not been associated with renal or auditory toxicity. Combination therapy with oxaliplatin and fluorouracil/calcium folinate appeared to be less well tolerated than fluorouracil-based therapy alone in terms of incidences of severe diarrhoea, vomiting and neutropenia; however, no significant difference in overall tolerability was reported.

Clinical trials have used a variety of dosages and administration schedules; however, the optimal treatment regimen remains to be determined, including the most effective combination therapy. A significantly higher response rate has been achieved by sequential administration of a 12-hour chronomodulated infusion of oxaliplatin followed by a 12-hour chronomodulated infusion of fluorouracil/calcium folinate than with a 24-hour fixed rate infusion of all 3 agents as first-line therapy in 2 studies. Furthermore, chronomodulated delivery of these agents appeared to be less toxic than continuous infusion.

Colorectal cancer is highly metastatic, the most common site being the liver. Many patients present with hepatic metastases that are considered unresectable because of their size, location and/or number. Prognosis of such patients is poor, with treatment options including cryosurgery and fluorouracil-based chemotherapy. Treatment with oxaliplatin and fluorouracil/calcium folinate has recently been found to reduce tumour size and facilitate the surgical removal of distant (particularly hepatic) metastases in patients whose tumours were previously unresectable. Available data indicate that the resection of previously unresectable tumours became possible in up to 16% of patients, and 3- and 5-year survival rates were 54 and 40%, respectively, with oxaliplatin and fluorouracil-based chemotherapy. No comparative studies have been conducted, but these results indicate that this treatment option is likely to be superior to fluorouracil-based therapy alone.[83]

Thus, oxaliplatin is a promising treatment option for patients with metastatic colorectal cancer. It appears to be particularly advantageous (in terms of response rate and duration of progression-free survival) when used in combination with fluorouracil/calcium folinate as both a first- and second-line option, although preliminary studies have failed to show any survival advantage over fluorouracil/calcium folinate alone. Promising results have been found in studies of the drug as monotherapy and oxaliplatin may also prove useful in the neoadjuvant setting in patients with unresectable liver metastases; however, data is limited at present.

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Exhibit C

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Advances in Brief

Nonconservative Amino Acid Substitution Variants Exist at Polymorphic Frequency in DNA Repair Genes in Healthy Humans[1]

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Abstract

The removal or repair of DNA damage has a key role in protecting the genome of the cell from the insults of cancer-causing agents. This was originally demonstrated in individuals with the rare genetic disease xeroderma pigmentosum, the paradigm of cancer genes, and subsequently in the relationship between mismatch repair and colon cancer. Recent reports suggest that individuals with less dramatic reductions in the capacity to repair DNA damage are observed at polymorphic frequency in the population; these individuals have an increased susceptibility to breast, lung, and skin cancer.

We report initial results from a study to estimate the extent of DNA sequence variation among individuals in genes encoding proteins of the DNA repair pathways. Nine different amino acid substitution variants have been identified in resequencing of the exons of three nucleotide excision repair genes (*ERCC1*, *XPD*, and *XPF*), a gene involved in double-strand break repair/recombination genes (*XRCC3*), and a gene functioning in base excision repair and the repair of radiation-induced damage (*XRCC1*). The frequencies for the nine different variant alleles range from 0.04 to 0.45 in a group of 12 healthy individuals; the average allele frequency is 0.17. The potential that this variation, and especially the six nonconservative amino acid substitutions occurring at residues that are identical in human and mouse, may cause reductions in DNA repair capacity or the fidelity of DNA repair is intriguing; the role of the variants as cancer risk factors or susceptibility alleles remains to be addressed.

Introduction

One of the early documented examples of genetic predisposition to cancer was the identification of the association of the rare cancer-prone condition xeroderma pigmentosum with defects in the nucleotide excision pathway for repairing DNA damage (1). Subsequently, defects in the process of mismatch repair of DNA were identified as a causative factor for familial colon cancer (2). Many studies have now documented that the genes involved in DNA repair and maintenance of genome integrity are critically involved in protecting against mutations that lead to cancer and/or inherited genetic disease (see reviews in Refs. 3–5).

Studies of inherited cancer or cancer families have resulted in the identification of an extensive number of cancer genes. Individuals with genetic variation resulting in loss of functionality for many of these cancer genes have a risk of cancer approaching unity (6–9). Even though an extensive number of cancer genes have been identified, the majority of cancer cases are sporadic rather than familial (2, 6, 10). Still, even in sporadic cancer cases, in the absence of other known risk factors, such as exposure to carcinogen or inheritance of a known cancer gene, the existence of a first-degree relative with cancer is a very significant risk factor (11–13). This suggests that genetic variation is a key element in susceptibility to cancer in most individuals, not only individuals in cancer families.

The genes associated with increased risk in sporadic cancer cases are referred to as "susceptibility" genes. Previous work to define the role of cancer susceptibility genes has often focused on variation in activity of the carcinogen-metabolizing enzymes. Molecular epidemiology studies have shown that variant alleles at several of these loci are associated with severalfold increases in cancer risk (14–16). As expected for susceptibility genes, these alleles are not highly penetrant, but the inheritance of genetic variants at one or more loci results in an increase in an individual's risk of cancer.

Interindividual variation in DNA repair capacity as measured with several lymphocyte assays has been observed, and individuals with a repair capacity of 65–80% of the population mean are more often in the cancer cohorts than in the control cohorts (17–25). Reduced DNA repair capacity constitutes a statistically significant risk factor for cancer, with odds ratios ranging from 1.6 to 10.0 in different studies and different cohorts, including breast and lung cancer (17–25). For comparison, cells from xeroderma pigmentosum patients exhibit a level of nucleotide excision repair capacity that is not significantly elevated over the experimental background activity of 1–2% of normal.

There is considerable evidence that DNA repair capacity is genetically determined. The phenotype of reduced repair capacity for one pathway, *e.g.*, nucleotide excision repair, is independent of the phenotype for another pathway, *e.g.*, double-strand break repair (23); this is consistent with repair capacity being genetically regulated. Twin studies support a genetic component in repair capacity (26). The elevated frequency of individuals with reduced repair capacity among relatives of cancer patients with reduced repair capacity also suggests that repair capacity is a genetic trait (19, 21, 22, 27). This variation in DNA repair capacity has characteristics expected of cancer susceptibility genes.

To support future molecular epidemiology studies that address the role of genetic variation at the genes of DNA repair in cancer susceptibility, we have initiated an effort to screen DNA repair genes for DNA sequence variation. We have focused on identifying variation causing amino acid substitutions and variation existing at polymorphic allele frequencies (allele frequencies >0.05). Given the known relationship of DNA repair to cancer, the polymorphic variants identified have the potential to be population cancer risk factors because of the large number of individuals affected.

We have selected five DNA repair genes, representing three different repair pathways, for this initial study. Current knowledge of the proteins in these repair pathways indicates that they function as members of multiprotein complexes, making it likely that amino acid residues at protein-protein interfaces, in addition to residues involved

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in the active site(s), will be important for protein function. Three of the genes, *XPD*, *XPF*, and *ERCC1*, belong to the nucleotide excision repair pathway and are members of a complex of 13–15 proteins that removes bulky adducts and thymidine dimers from DNA by excising a 24–32 nucleotide single-strand oligomer containing the adduct (28). XPD functions as an ATP-dependent 5′–3′ helicase (29) within the basal transcription factor IIH complex, whereas XPF and ERCC1 form a complex that incises DNA at the 5′ side of a bulky adduct lesion. The XPF and ERCC1 proteins are also known to interact with the RPA and XPA proteins (30). The fourth gene, *XRCC1*, was originally isolated as a radiation-sensitive mutant and assigned to the double-strand break/recombination pathway of DNA repair (31). Recent biochemical characterization has identified XRCC1 interaction with DNA POLB,[3] PARP, and DNA ligase III (32, 33), suggesting a role for XRCC1 in the base excision repair pathway, although a specific function for XRCC1 has not been identified (28). Domains of XRCC1 that make contact with the proteins of the base excision repair pathway have been identified. A BRCT domain, a domain found in many proteins with cell cycle checkpoint functions and responsive to DNA damage (34), has also been identified in XRCC1. The fifth gene, the recently identified *XRCC3*, is a RAD51 homologue.[4] XRCC3 participates in DNA double strand break/recombination repair, but little is known about its specific function (31). We report here the identification of nine different amino acid substitutions, existing at an average allele frequency of 0.17, in resequencing five DNA repair genes from 12 healthy individuals.

Table 1 *Primers for PCR amplification of genomic DNA*

Gene[a]	Exons	Primers	Sequences
XRCC1	4	Rex4s-Xr1	R-CCCTTGCCTTTCCGCTGAC
		Fex4a-Xr1	F-AGTTCCCCTCCTCCGATTC
	5 and 6	Rex5.6s-Xr1	R-GCCAGGGCCCCTCCTTCAA
		Fex5.6a-Xr1	F-TACCCTCAGACCCACGAGT
	7 and 8	UFex7.8s.Xr1	F-GTCCCATAGATAGGAGTGAAAG
		URex7.8a2.Xr1	R-CCCTAGGACACAGGAGCACA
	9 and 10	URex9.10s-Xr1	R-CAGTGGTGCTAACCTAATC
		UFex9.10a-Xr1	F-AGTAGTCTGCTGGCTCTGG
	11 and 12	Fex11.12s2-Xr1	F-CCTTGGGCCTGTTTGTCTGA
		Rex11.12a3-Xr1	R-TCCTCCCTCAGAGTCTGACC
	13 and 14	URex13.14s2-Xr1	R-GGATCTGGAGGGCAGTTGAG
		UFex13.14a-XR1	F-CCCAGCTGAGAACTGAGAA
	16	Rex16.17s-Xr1	R-GAGTGGCTGGGGAGTAGGA
		Fex16.17a-Xr1	F-GCCAAGCAGAAGAGACAAA
XRCC3	1	Fex1s-Xr3	F-GGGAGGAGGTCGTCGCTAAA
		Rex1a-Xr3	R-AGGCAGCCTGGGGAGTATGA
	4	Fex4s2-Xr3	F-GGCTGGTATCTGTCCGAGTG
		Rex4a2-Xr3	R-CACGCATCTTCTGACCCGAT
	7	Fex7s-Xr3	F-GGTCGAGTGACAGTCCAAAC
		Rex7a-Xr3	R-CTACCCGCAGGAGCCGGAGG
ERCC1	3	Fex3s-Er1	F-CCTCAGATGTCCTCTGCTCA
		Rex3a-Er1	R-GCCACAGCCCCAGCAAGTAG
	4	Fex4s2-Er1	F-AGGACCACAGGACACGCAGA
		Rex4a2-Er1	R-CATAGAACAGTCCAGAACAC
	5	Fex5s-Er1	F-GCCCTTAGTATTCCAGTGAG
		Rex5a-Er1	R-GGACTAATTGAAGGGGATGT
	6	Fex6s-Er1	F-TTTGTAATTCCTGGCTTCTA
		Rex6a-Er1	R-GACCTTGTTTTACAGATGAG
	10	Fex10s-Er1	F-TAAATGCTTGAGGGTATAGG
		Rex10a-Er1	R-GCCGGGACAAGAAGCGGAAG
XPD	5	Fex5s-Er2	F-CCAGCTTTCGGGGGTGTTTG
		Rex5a-Er2	R-AATGAGAATTTGACCACTGA
	6 and 7	Fex6.7s-Er2	F-AGACCAGGGTTTGAAGAGTG
		Rex6.7a-Er2	R-CTCACAGCAAGCAACAGACA
	8 and 9[b]	Fex8.9s-Er2	F-GGCCTGTGTGGGAGTGACGG
		Rex8.9a2-Er2	R-CTGCTCGTCTGTCTCTTTGA
	10 and 11[b]	Fex10.11s2-Er2	F-TGACCGGTGCCAGGGCAACC
		Rex10.11a-Er2	R-GGACACGGCTCTGCATAACC
	17	Fex17s-Er2	F-AAACTCCTAGTTCTAAGACA
		Rex17a-Er2	R-TGCTTACACCCCATTCCTAC
	18 and 19	Fex18.19s-Er2	F-CAGAAGAGTTGGATGTAACC
		Rex18.19a-Er2	R-GCGGGAGCAGACAGCAGAGC
	20 and 21	Fex20.21s-Er2	F-CAACTCAGACACAGCATCCT
		Rex20.21a-Er2	R-ACTCTCCACCCTGCAACCCA
	22	Fex22s-Er2	F-GGCTGTTTCCCGTTCATTTC
		Rex22a-Er2	R-GTAGATGCACGATAAACTTC
	23	Fex23s-ER2	F-TCAAACATCCTGTCCCTACT
		Rex23a-Er2	R-CTGCGATTAAAGGCTGTGGA
XPF	1	Fex1s-Er4	F-CACGATCATCTCAGTCTCAG
		Rex1a-Er4	R-TCCTCCTAGCGACCCCTTAC
	7	Rex7s-Er4	R-ATATGTACTGATGCTCGTGT
		Fex7a-Er4	F-CTAGGATCTCAGTGTTCATT
	10	Fex10s-Er4	F-TTTCTCTTACTGCTATCATC
		Rex10a-Er4	R-AAGTACACATCCTCTCCTTG
	11.2	Fex11s2-Er4	F-TCTCCATGTCCCGCTACTAC
		Rex11a2-Er4	R-GCAGGCACAGGCAAGTTCAA

[a] The GenBank accession numbers for the genes are: *XRCC1*, L34079; *ERCC1*, M63796; *XPD* (*ERCC2*), L47234; *XPF* (*ERCC4*), L76568; and *XRCC3*, GSDB:S:1297788.

[b] DMSO (5%) was required in these PCR reactions.

Table 2 *Summary of single-nucleotide polymorphisms that do not result in an amino acid substitution*

Gene	Segment	Allele frequency	Nucleotide		Amino acid residue
			Position	Variation	
XRCC1	Intron 3	0.25	24737	TGGGGC/ACTGTG	
XRCC1	Intron 6	0.08	26350	GTCCTG/AAGTGA	
XRCC1	Intron 6	0.08	26602	CAGCCC/TTCTCA	
XRCC1	Exon 7	0.42	26651	GACCCA/GGCAGG	206 Pro
XRCC1	Intron 9	0.42	27826	TCACTT/CGCTTT	
XRCC1	Intron 9	0.25	27980	GTCTCA/GTTCCC	
XRCC1	Intron 11	0.17	32772	CCCCAA/GACTTC	
XRCC1	Intron 13	0.42	33543	GGGCTG/AGGGCT	
XRCC1	Exon 17	0.42	36189	CCGCAG/AGCCTG	632 Gln
XPD	Intron 4	0.21	18814	AATGAA/GCACAA	
XPD	Intron 5	0.04	18980	TCCTTC/TTCCTT	
XPD	Exon 6	0.25	22541	TGCCGC/ATTCTA	156 Arg
XPD	Intron 6	0.04	22559	ACTGGA/CGGGCA	
XPD	Intron 7	0.33	22812	CCGCCC/TGTCTC	
XPD	Intron 17	0.04	32983	GAGTGC/TGTGCA	
XPD	Intron 18	0.29	34382	GGGGTG/CGGGGA	
XPD	Intron 19	0.25	34706	TGGGTC/TGCGTG	
XPD	Intron 19	0.04	34750	CCCCCC/TTCGCC	
XPD	Intron 19	0.04	34770	CTGCCCC/TACCAG	
XPD	Exon 22	0.25	35326	GTGGAC/TGAGGG	711 Asp
XPD	Intron 22	0.04	35788	CATTC(G)GGGGG	Insertion
XPD	Intron 22	0.29	35790	TTCGGG/CGGGGT	
XPF	5′-UTR[a]	0.46	2063	TCGGCT/AGCGTT	
XPF	Intron 1	0.46	2310	GCCGCG/ACTGGC	
XPF	Intron 1	0.04	2348	TGAGGG/ACCTCC	
XPF	Intron 9	0.38	26615	AAATTT/CGTTTC	
XPF	Intron 9	0.38	26622	TTTCAG/AAAGTG	
XPF	Exon 11	0.38	30028	GATTCT/CGAAAC	824 Ser
XRCC3	5′ region	0.21	4541	GCAGGA/GTGTGC	
XRCC3	Intron 4	0.04	8995	GGTGAC/TATGTG	
XRCC3	Intron 6	0.38	17893	ATGACA/GGCTGT	
ERCC1	Exon 3	0.04	19886	GGGCAC/TGTGGC	75 Thr
ERCC1	Intron 3	0.50	19716	TCCTTG/CCACTG	
ERCC1	Exon 4	0.46	19007	GGCACG/ATTGCG	118 Asn
ERCC1	Exon 5	0.04	17773	CGCCCA/GTGGAT	154 His
ERCC1	Intron 5	0.33	17677	AGCCCG/TGTGAG	
ERCC1	Intron 6	0.13	15310	CCAGAG/CAGAGG	
ERCC1	3′UTR	0.04	8092	GGAAGC/AAGCAG	

[a] UTR, untranslated region.

Materials and Methods

PCR Amplification Conditions. The PCR primers were designed using the Oligo Primer Analysis Software (National Biosciences, Inc., Plymouth, MN) and usually directed to intronic or noncoding sequences ~50 bp away from exon/intron boundaries. Appended to the 5′ end of each of the PCR primers were sequences containing the primer binding sites for the forward or reverse energy transfer DNA sequencing primers (Amersham Life Science, Cleveland, OH). PCR primers were matched so that the sense and the antisense PCR primers contained different sequencing primer binding sites. PCR primers were tested under a single thermocycle condition and optimized by addition of DMSO or $MgCl_2$. PCR primers that could not be

[3] The abbreviations used are: POLB, polymerase β; PARP, poly(ADP-ribose) polymerase; BRCT, breast cancer COOH terminus; ABI, Applied Biosystems, Inc.

[4] N. Liu, J. Lamerdin, and L. H. Thompson, unpublished data.

Table 3 *Summary of amino acid substitution variants observed in resequencing of five DNA repair genes*[a]

Gene	Exon	Nucleotide substitution		Allele frequency	Amino acid	
		Position	Change[b]		Position	Change
XRCC1	6	26304	TCAGCC/TGGATC	0.25	194	Arg-Trp
XRCC1	9	27466	AACTCG/ATACCC	0.08	280	Arg-His
XRCC1	10	28152	CTCCCG/AGAGGT	0.25	399	Arg-Gln
XPD	8	23047	CAGATC/GCTGCA	0.04	199	Ile-Met
XPD	8	23051	TCCTGC/TATGCC	0.04	201	His-Tyr
XPD	10	23591	TGCCG/AACGAA	0.42	312	Asp-Asn
XPD	23	35931	CGCTGA/CAGAGG	0.29	751	Lys-Gln
XRCC3	7	18067	GGCCAC/TGCTGC	0.38	241	Thr-Met
XPF	7	16151	GCAACC/TCAAAG	0.08	379	Pro-Ser

[a] No variants were identified in *ERCC1*.
[b] The variant residues are underlined, with the common nucleotide followed by the variant.

optimized to perform under these conditions were redesigned. PCR reactions were performed in a 50-μl reaction volume using a hot-start format. The final components of the reaction were as follows: 1× PCR buffer [10 mM Tris-HCl (pH 8.3; 20°C), 1.5 mM $MgCl_2$, and 50 mM KCl], 200 mM each deoxynucleotide triphosphate, 0.5 μM each primer, 1.25 units of Taq DNA polymerase (Boehringer Mannheim), and 50 ng of genomic DNA. For the hot-start format, all of the reaction components except for Taq DNA polymerase were combined in a 40-μl volume. The reactions were placed into a Perkin Elmer 9600 GeneAmp thermocycler and subjected to the following thermocycle conditions: initial denaturation at 94°C for 5 min (during which time the Taq DNA polymerase in a 10-μl volume of 1× PCR buffer was added to the reaction mix), followed by 35 cycles of denaturation at 94°C for 30 s, primer annealing at 63°C for 45 s, and primer extension at 72°C for 60 s; a final incubation at 72°C for 7 min was performed. PCR products were analyzed in a 2% agarose gel.

PCR primer sequences for amplification of the fragments in which sequence variation was identified are in Table 1. Primer sequences for amplification of the remaining exons can be obtained by contacting the corresponding author.

DNA Sequencing. For most of the resequencing, the PCR products were diluted 10-fold with TLE [10 mM Tris-HCl (pH 8.0; 20°C) and 0.1 mM EDTA] and used directly in sequencing reactions. PCR products were sequenced in both directions; heterozygotes detected in one strand were confirmed in the opposite strand. Sequencing reactions were performed according to the manufacturer's instructions using the DYEnamic Direct cycle sequencing kit with the DYEnamic energy transfer primers (Amersham Life Science, Inc., Cleveland, OH). The thermocycle conditions for the cycle sequencing reactions were 25 cycles of 95°C for 30 s, 50°C for 5 s, and 72°C for 60 s. The pooled precipitated sequencing products were resuspended in 6 μl of the supplied loading buffer and heat denatured, and 2.5 μl were loaded into an ABI Prism 373 stretch DNA sequencer (Foster City, CA).

In early resequencing, the PCR product was digested with exonuclease I and calf intestinal alkaline phosphatase to degrade excess primers and deoxynucleotide triphosphates (35). It was found that high quality sequence was obtained without inclusion of the treatment step; this step was not utilized for generating most of the data accumulated.

DNA Sequence Analysis. The initial data analysis (lane tracking and base calling) was performed with the ABI prism DNA sequence analysis software (version 2.1.2). Chromatograms created by the ABI prism DNA sequence analysis software were imported into a Sun Microsystems Unix workstation (Sun Microsystems Inc., Mountain View, CA). The chromatograms were reanalyzed with Phred (bases were called and quality values were assigned; version 0.961028) and assembled with Phrap (version 0.960213), and the resultant data were viewed with Consed (version 4.1).[5]

Samples. DNA for PCR amplification was isolated from archived placenta or lymphocytes by standard techniques. The samples were from unidentified individuals, and no characteristics of the individuals are known, although they are presumed to have been healthy at the time of sample collection. Because the samples cannot be associated with a donor, they were deemed to be exempt by the Institutional Review Board.

[5] Description and documentation for Phred, Phrap, and Consed may be obtained at http://www.genome.washington.edu.

Results and Discussion

Nucleotide Substitutions in Noncoding Regions. Although the focus of this effort was the resequencing of exons to identify amino acid substitutions of potential functional significance, the strategy of using PCR amplification of genomic DNA to generate products for sequencing means that some intronic regions were also resequenced. The summary of the DNA sequence variation observed in the resequencing of intronic regions of the five DNA repair genes in 12 individuals is presented in Table 2. Twenty-six different nucleotide substitution variants and one single nucleotide insertion variant were identified in intronic sequences. None of the substitutions destroyed a splice site or generated an obvious cryptic splice site.

Approximately 100 nucleotides at the 5′ and 3′ ends of each gene were also scanned for variation. As seen in Table 2, one substitution was detected 5′ of the translation initiation codon of the *XRCC3* gene, one substitution was identified in the 5′ region of *XPF*, and another substitution was identified at the 3′ end of *ERCC1*. None of these substitutions occurred in known regulatory elements. In total, 30 different variants existing in 159 copies were identified during the resequencing of 334 kb of nonexonic DNA (13.9 kb per chromosome × 12 individuals × 2 chromosomes per person). Thus, a nucleotide substitution variant was observed every 2.1 kb of noncoding DNA resequenced.

Nucleotide Substitutions in Exons. Resequencing of 224 kb of exonic DNA resulted in identification of 17 different nucleotide substitutions and a total of 98 variant alleles. This is a variant allele every 2.3 kb of DNA, a frequency of nucleotide substitution that is very similar to the frequency observed in introns.

Eight nucleotide substitutions that did not result in amino acid substitutions were identified (also included in Table 2). None of the substitutions involved splice sites, and therefore, except for the potential to impact protein synthesis through generation of rare and/or underutilized codons, these substitutions should not impact protein function. The silent nucleotide substitutions at the Arg 156 and Asp 711 codons of *XPD* have been observed previously at similar frequencies in studies from England (36).

Amino Acid Substitution Variants. Nine amino acid substitution variants were identified during the resequencing of exons from 12 healthy individuals (Table 3). The variants were detected in four of the five genes screened; no amino acid substitution variants were identified in resequencing of *ERCC1*. An average of 1.8 unique or different variants per gene (nine variants/five genes) was identified during the resequencing of exons from the 12 presumably healthy individuals studied. The nine variant alleles existed in frequencies ranging from 0.04 (1 variant detected in the sample of 24 chromosomes) to 0.42 (10 variants in the sample of 24 chromosomes; Table 3); the average allele frequency for these nine amino acid substitution variants is 0.17. A

Table 4 *Conservation of amino acid residues at sites of variation*

Gene	Amino acid		Mouse sequence	Hamster sequence	Fish sequence
	Position	Change[a]			
XRCC1	194	PhePheSer(Arg-Trp)IleAsnLys	Arg	Arg	NA[b]
XRCC1	280	AlaProThr(Arg-His)ThrProAla	Arg	Arg	NA
XRCC1	399	LeuProSer(Arg-Gln)ArgTyrLeu	Arg	Arg	NA
XPD	199	ArgTyrSer(Ile-Met)LeuHisAla	Ile	Ile	Ile
XPD	201	SerIleLeu(His-Tyr)AlaAsnVal	His	His	His
XPD	312	ValLeuPro(Asp-Asn)GluValLeu	Asp	Asp	Asp
XPD	751	GluThrLeu(Lys-Gln)ArgIleGlu	Gln	Arg	Gln
XRCC3	241	LeuGlyAla(Thr-Met)LeuArgGlu	NA	NA	NA
XPF	379	GluSerAsn(Pro-Ser)LysTrpGlu	Pro	NA	NA

[a] The common amino acid residue in human is followed by the variant residue within parentheses.
[b] NA, sequence not available.

nucleotide substitution resulting in an amino acid substitution was detected every 5.1 kb of exonic DNA resequenced. The substitutions of Asp to Asn at position 312 and Lys to Gln at position 751 of XPD were identified previously at allele frequencies of ~0.5 and 0.30 in a report from England (36).

Given the high allele frequencies, it was not surprising to observe homozygous individuals, even in this small sample. One individual was homozygous for the *R194W* variant in exon 6 of *XRCC1*. Another individual was homozygous for the *D312N* variant allele in exon 10 of *XPD* and also heterozygous for the *K751Q* variant in exon 23. In addition, this individual was heterozygous for the *P379L* variant in exon 7 of *XPF*. Thus, in this individual, all of the excision repair complex protein molecules would contain a variant form of the XPD protein; 50% of the molecules would have an XPD subunit with two amino acid substitutions, and half of the molecules would contain variant subunits of both XPD and XPF.

The data suggest that certain alleles exist on the same chromosome and form a haplotype, although genetic transmission data are necessary to confirm the linkage. For example, the amino acid substitution variant *R399Q* in exon 10 of *XRCC1* and the nucleotide substitutions *C24737A* and *A27920G* in introns 3 and 9 of *XRCC1* were always (and only) identified in the same six individuals.

Characteristics of Amino Acid Substitutions. Seven of the nine amino acid substitutions are nonconservative replacements, the exceptions being Arg/Gln at position 399 of *XRCC1* and Lys/Gln at position 751 of *XPD*. Six of the seven nonconservative substitutions occur at amino acid residues that are known to be identical in the human and mouse genes; the possible exception is *XRCC3*, in which the sequence of mouse *XRCC3* is currently not known.

The amino acid substitutions in XPD do not reside in known or hypothesized helicase/ATPase domains. However, three of the four amino acid changes are nonconservative substitutions (Table 4), the exception being the *K751Q* variant, and the nonconservative substitutions are at amino acid residues that are identical in human, mouse, hamster (37), and fish XPD (38). Thus, the amino acid substitutions in XPD that have been identified in the screen of this healthy human population have occurred at residues that are highly conserved through evolution. This sequence conservation is indicative of a functional role for these residues. None of the amino acid substitutions found at polymorphic frequency are among the amino acid substitutions of functional domains of XPD that have been associated with significant loss of function or any of the three genetic diseases assigned to this locus, including the cancer-prone condition xeroderma pigmentosum (39, 40). This is as expected, given the rarity of the diseases, which contrasts with the polymorphic frequency of the alleles identified via resequencing.

The three amino acid substitutions in XRCC1 occur at residues that are identical in hamster and human[6] and mouse (41). Two of the variants (R194W and R280H) reside in the linker regions separating the DNA POLB domain from the PARP-interacting domain (40, 41). The R194W change is a nonconservative substitution occurring within a hydrophobic core. The R399Q change resides at the COOH-terminal side of the PARP-interacting domain and within an identified BRCT domain. The R399Q substitution is within a relatively nonconserved region between conserved residues of the BRCT domain. The R280H variant is another nonconservative substitution. Single amino acid substitutions in both the BRCT domain and in the DNA POLB-interacting regions in the hamster XRCC1 have been shown to completely disrupt the functionality of the XRCC1 protein.[6] The absence of XRCC1 activity in the mouse is an embryo-lethal condition (42). Thus, it is assumed that the variant alleles identified in this resequencing screen do not cause complete loss of protein function. The evolutionary conservation of the residues among species would suggest some functional significance for these residues in the maintenance of normal protein function.

Less is known about the functional domains of XRCC3 and XPF. The T241M substitution in XRCC3 is a nonconservative change, but it does not reside in the ATP-binding domains, which are the only functional domains that have been identified in the protein at this time.[4] The single nonconservative substitution in XPF (P379S) is at a residue that is identical in humans and mice.[7]

This preliminary study of variation at five loci encoding DNA repair proteins found that nonconservative amino acid substitution variants exist at polymorphic frequency at four of the five loci screened. An average of 1.8 different variant alleles per locus were identified in screening only 12 healthy individuals; the average frequency for each of the nine variant alleles was 0.17. Thus, these are common variant alleles. Therefore, if of functional significance, these variants exist in frequencies sufficient to have significant health consequences for the population.

The finding that none of the variation exists in known functional domains of these proteins is not surprising, given that known amino acid substitutions in these domains cause loss of function and disease or embryo lethality (1, 42) and thus are under negative selective pressure. The observation that most of the amino acid substitutions identified in this study are at residues that are conserved through evolution, however, suggests that these residues are important in maintaining normal protein structure and integrity and that the amino acid substitutions could result in a protein with reduced function in either repair capacity or fidelity. Biochemical and biological characterization of these variants, especially the nonconservative amino acid

[6] M. R. Shen, M. Z. Zdzienicka, H. Mohrenweiser, L. H. Thompson, and M. P. Thelen, Mutations in hamster XRCC1 causing defective repair of single-strand breaks, Nucleic Acids Res., in press, 1998.
[7] M. Shannon and M. P. Thelen, unpublished data.

substitutions, and molecular epidemiology studies in cancer case and control cohorts will provide insight into the potential for these variants to be cancer susceptibility alleles.

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